MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

Vol. XLVI MARCH-APRIL, 1954 No. 2

CONTENTS

Mechanism of action of copper 8-quinolinolate.... RICHARD E. VICKLUND, MILTON MANOWITZ AND VINCENT J. BAGDON 133

The sensitization of ascospores to chemical activators by heat treatment..... ALFRED S. SUSSMAN 143

Spore germination in Ustilago zeae as influenced by KH_2PO_4 THOR KOMMEDAHL 151

The parasitic growth phase of Coccidioides immitis in culture N. F. CONANT AND R. A. VOGEL 157

Studies of the creosote fungus, Hormodendrum resinae DAVID H. MARSDEN 161

Culture studies in the genera Pleospora, Clathrospora and Leptosphaeria. II..... Emory G. Simmons 184

Sexual reproduction in Gonapodya ROBERT M. JOHNS AND R. K. BENJAMIN 201

Taxonomic studies in the genus Coccospora SAMUEL C. DAMON AND MARY H. DOWNING 209

Morphology of spore forms and heteroecism in Puccinia cacao M. J. THIRUMALACHAR AND M. J. NARASIMHAN 222

Polyporus montagnei and Cyclomyces greenei ROBERT L. GILBERTSON 229

Poria cocos, a widely-distributed wood-rotting fungus ROSS W. DAVIDSON AND W. A. CAMPBELL 234

Observations on Tilletia cerebrina..... W. N. SIANG 238

A new species of Comatricha from Jamaica CONST. J. ALEXOPOULOS AND E. S. BENEKE 245

The genus Cordyceps and Fries's Observationes DONALD P. ROGERS 248

Notes and Brief Articles..... 254

Reviews..... 257

[MYCOLOGIA for January–February (46: 1–132) was issued February 16, 1954]

PUBLISHED BIMONTHLY FOR

THE NEW YORK BOTANICAL GARDEN

AT PRINCE AND LEMON STS., LANCASTER, PA.

Entered as second-class matter April 30, 1925, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

MYCOLOGIA

Published by

THE NEW YORK BOTANICAL GARDEN

IN COLLABORATION WITH THE

MYCOLOGICAL SOCIETY OF AMERICA

OFFICERS OF THE MYCOLOGICAL SOCIETY OF AMERICA

LELAND SHANOR, *President*
University of Illinois

DONALD P. ROGERS, *Vice-President*
New York Botanical Garden

C. J. ALEXOPOULOS, *Sec.-Treas.*
Michigan State College

J. L. LOWE, *Councilor*, 1953–54
New York State College of Forestry

J. C. GILMAN, *Councilor*, 1953–54
Iowa State College

LINDSAY S. OLIVE, *Councilor*, 1954–55
Columbia University

RALPH EMERSON, *Councilor*, 1954–55
University of California

EDITORIAL BOARD

G. W. MARTIN, *Editor-in-Chief*
State University of Iowa
Box 324, Iowa City, Iowa

DONALD P. ROGERS, *Managing-Editor*
New York Botanical Garden
Bronx Park, New York 58, N. Y.

ALMA J. WHITTEN, '54
New York Botanical Garden
Bronx Park, New York 58, N. Y.

EDITH K. CASH, '55
Plant Industry Station, Beltsville, Maryland

C. W. EMMONS, '56
National Institute of Health
Bethesda 14, Maryland

F. L. DRAYTON, '57
Department of Agriculture, Ottawa, Canada

H. L. BARNETT, '58
West Virginia University, Morgantown, W. Va.

SUSTAINING MEMBERS

Abbott Laboratories
The American Sterilizer Company
Baltimore Biological Laboratory, Inc.
Ben Venue Laboratories, Inc.
Beckman Laboratories, Inc.
Difco Laboratories
E. I. Dupont de Nemours & Company
Hayden Chemical Corporation
Lederle Laboratories
Chas. Pfizer & Co., Inc.
E. R. Squibb and Sons
Standard Brands, Inc.
The Arthur H. Thomas Company
The Wallerstein Company

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

XLVI MARCH–APRIL, 1954 No. 2

MECHANISM OF ACTION OF COPPER 8–QUINOLINOLATE

RICHARD E. VICKLUND,[1] MILTON MANOWITZ [2] AND VINCENT J. BAGDON

(WITH 1 FIGURE)

Copper 8-quinolinolate has been found to be outstanding for inhibiting the mildewing and rotting of various materials. Several publications demonstrate its effectiveness as a textile preservative (**2**, **6**, **7**); its protective value in paints and varnishes was reported (**3**), and in collateral publications the authors show that its fungicidal activity compares very favorably with that of the fungicides most frequently used in textiles, paints and varnishes. Still other reports indicate that it prevents the mildewing and rotting of leather, plastics and wood.

While these results show that the activity of copper 8-quinolinolate is of a high order, it is especially significant that the compound is effective against many fungi in a wide variety of materials of entirely different chemical composition. In many cases, a fungicide is rather specific in the type of material in which it is effective; for example, copper naphthenate and 2,2′-methylenebis (4-chlorophenol) are effective textile preservatives but are of no value in preventing the mildewing of paints. This may be due to the fact that there are many modes of action by which the growth of microorganisms can be inhibited or their effects minimized; in

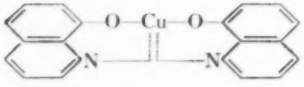
[1] Formerly of the Engineer Research and Development Laboratories, Fort Belvoir, Virginia, now at Sindar Corporation, 330 West 42nd Street, New York 16, New York.

[2] Formerly of the Engineer Research and Development Laboratories, Fort Belvoir, Virginia, now at the Biological Laboratories, Quartermaster Depot, Philadelphia, Pennsylvania.

[MYCOLOGIA for January–February (**46**: 1–132) was issued February 16, 1954]

some of these mechanisms the composition of the material under attack plays a significant role and when the material is changed the mechanism may be disrupted. It is also possible that other physical and chemical influences that the material and fungicide exert upon each other, such as solubility and compatibility, account for the limitations upon the effectiveness of a fungicide in protecting various materials. However, copper 8-quinolinolate appears to function by some means which is independent of the material and disrupting to some vital metabolic process common to numerous genera of fungi.

In addition to its high fungistatic activity, the physical and chemical properties of copper 8-quinolinolate are favorable from the practical viewpoint of achieving long-term protection under severe conditions. Many of these properties result from its inner complex chelate structure, which is shown below:



Benignus (**2**) has reported that the compound has a water solubility of 0.8 ppm and the vapor pressure is practically nil; it is insoluble in most organic solvents, non-hygroscopic, practically odorless, stable at 204° C, and also stable against the action of ultra-violet light and other actinic rays. Its color, yellow-green, and its insolubility in organic solvents place some limitations on its use; however, the authors and others (**3**) have succeeded in colloidally dispersing the compound, thus reducing this disadvantage.

Copper 8-quinolinolate is prepared from the reaction between 8-hydroxyquinoline and a copper salt such as copper acetate. This reaction is sometimes used to precipitate the copper complex in a textile, the textile being first passed through a solution of the 8-hydroxyquinoline followed by immersion in a solution of the copper salt. 8-hydroxyquinoline is commonly used as an analytical reagent for metal precipitation (**4**) and has been found to inhibit the growth of bacteria (**1**) and fungi (**10**). Its effectiveness as a fungicide, however, is considerably less than that of its copper complex.

THEORY OF MECHANISM OF ACTION

Observations made during various biological tests on copper 8-quinolinolate permitted the formulation of a hypothesis concerning its mechanism of action. It is suggested that copper 8-quinolinolate must first dissociate to form 8-hydroxyquinoline and copper ions, each of which

performs different but uniquely related functions. The 8-hydroxyquinoline inhibits the growth of fungi by precipitating those metals which are required for the metabolism of the organism. The copper ions function synergistically with the 8-hydroxyquinoline.

TEST PROCEDURE

Evidence in support of the above theory of the mechanism of action resulted from the analysis of test results which demonstrated that the fungistatic activity of both 8-hydoxyquinoline and copper 8-quinolinolate could be reversed by the addition of metal ions to the culture medium in which the compounds were tested. In these tests the active agent was mixed into an agar medium which, after hardening in Petri plates, was spot inoculated in the center with a water suspension of *Aspergillus niger* spores. The culture medium consisted of the following:

KH_2PO_4	2 gms
NH_4NO_3	3 gms
KCl	0.5 gms
Dextrose	30 gms
Agar	20 gms
Distilled water to make one liter pH adjusted to 5.5	

After a 96-hour incubation period the diameter of the fungus colony on each plate was measured and the per cent of inhibition, as compared with the colony in a plate which had no active agent present, was calculated. In order to produce the reversals of fungistatic activity, varying amounts of several metal salts were added to the above medium in addition to the 8-hydroxyquinoline and copper 8-quinolinolate. The results obtained are summarized in TABLE I; FIG. 1 shows the test plates in which the activity of the copper 8-quinolinolate was reversed.

THE DISSOCIATION OF COPPER 8-QUINOLINOLATE

The above results establish that portion of the mechanism of action theory which states that copper 8-quinolinolate must first dissociate to form 8-hydroxyquinoline and copper ions. It is shown that the addition of 50 ppm of ferrous sulfate to the medium containing a 100 percent effective concentration (1.5 ppm) of 8-hydroxyquinoline reduces the inhibition to 12 percent; the addition of sufficient copper ions to the same medium also reduces the activity. Since excess amounts of metal ions reverse the activity of 8-hydroxyquinoline, it follows that the activity of the copper 8-quinolinolate would also be reversed by the addition of

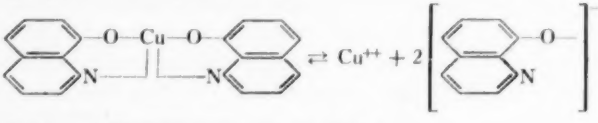
metal ions, provided the assumption that the copper salt dissociates to form 8-hydroxyquinoline and copper ions is correct. This was found to be the case; the addition of iron or copper ions to the medium reversed the activity of the copper 8-quinolinolate.

TABLE I

REVERSAL OF ACTIVITY OF 8-HYDROXYQUINOLINE AND COPPER 8-QUINOLINOLATE

Compound	Conc. ppm	Inhib. %	Compound	Conc. ppm	Inhib. %
8-hydroxyquinoline	1.0	0	Copper 8-quinolinolate	0.5	0
8-hydroxyquinoline	1.5	100	Copper 8-quinolinolate	1.0	100
8-hydroxyquinoline plus $CuSO_4$	1.5 92	100	Copper 8-quinolinolate plus $CuSO_4$	1.0 46	94
8-hydroxyquinoline plus $CuSO_4$	1.5 184	74	Copper 8-quinolinolate plus $CuSO_4$	1.0 92	65
8-hydroxyquinoline plus $FeSO_4$	1.5 50	12	Copper 8-quinolinolate plus $CuSO_4$	1.0 138	45
$CuSO_4$	46	0	Copper 8-quinolinolate plus $FeSO_4$	1.0 50	12
$CuSO_4$	92	10	Copper 8-quinolinolate plus $FeSO_4$	1.0 100	6
$CuSO_4$	138	31	$FeSO_4$	50	0
$CuSO_4$	184	44	$FeSO_4$	100	0

This dissociation of copper 8-quinolinolate may be expressed by the following equilibrium equation:



for which the equilibrium constant would be

$$K = C_x \times C_y$$

where C_x equals the concentration of the quinolinolate radical or possible 8-hydroxyquinoline and C_y equals the concentration of the metal ions. When the value of C_y is increased by the addition of excess metal ions to the medium the concentration of the 8-hydroxyquinoline must be correspondingly decreased. Thus, if large excesses of metal ions are present, the fungus can grow even though copper 8-quinolinolate is present in sufficient concentration to prevent growth in the original culture medium.

Maley and Mellor (**8**) have reported on the relative stability of metal salts of 8-hydroxyquinoline and give a quantitative treatment of the chelation of 8-hydroxyquinoline, based on the theory of step equilibria.

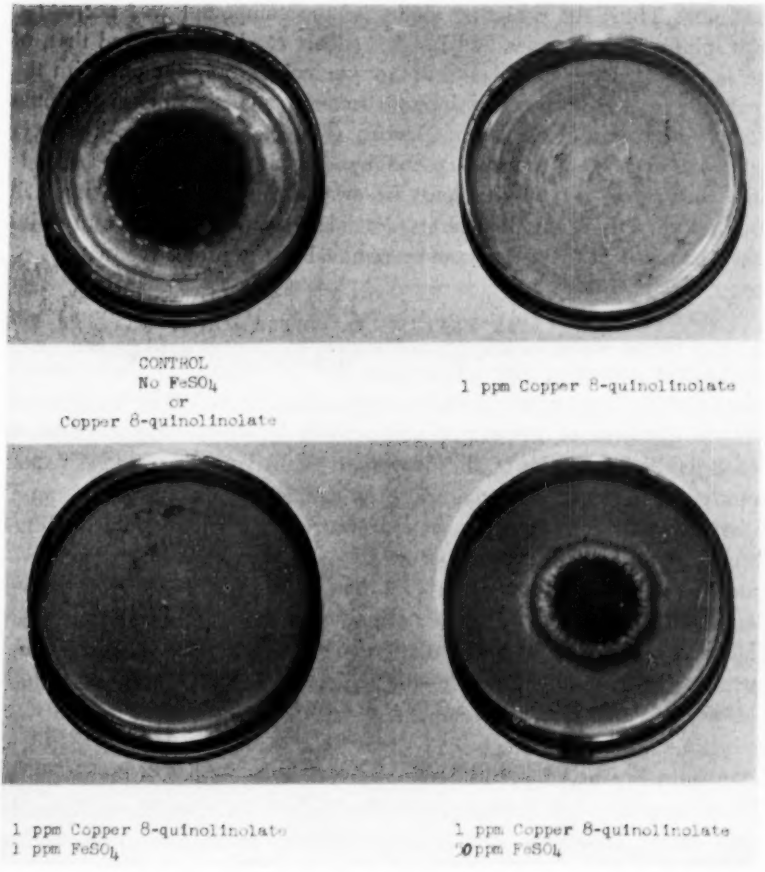


FIG. 1. Results of agar toxicity tests. Note that the fungicidal activity of copper 8-quinolinolate is reduced by the addition of Fe ions to the medium.

MECHANISM OF ACTION OF 8-HYDROXYQUINOLINE

The reversals of the activity of 8-hydroxyquinoline also indicate its mechanism of action. When an excess of metal ions is present the 8-hydroxyquinoline is largely present as the metal quinolinolate and cannot precipitate the metals which are required for growth of the organism. Both Albert *et al.* (**1**) and Zentmeyer (**13**) demonstrated that the activity of 8-hydroxyquinoline was reduced by incorporating increasing amounts of metal ions, such as iron and zinc, into the culture

medium. Thus, the chelating ability of the compound was accredited with inhibition of bacteria and fungi. Albert *et al.* also showed that, of the seven possible isomerides of hydroxyquinoline, only the one with the hydroxyl in the 8 position inhibited bacterial growth; this is the only isomeride capable of chelation. Rubbo (**11**) claimed that "8-hydroxyquinoline exerts antibacterial action by directly chelating with metals (prosthetic groups) of vital enzyme systems in the metabolizing cell and by indirectly promoting the irreversible oxidation of protein moieties (thiol groups) of these enzymes by removal of the protective metal."

THE FUNCTION OF COPPER

The data presented also substantiate that portion of the theory which states that the copper ions function synergistically with 8-hydroxyquinoline; the presence of copper produces activity far in excess of the sum of the individual activities of the copper and 8-hydroxyquinoline. TABLE I shows that copper 8-quinolinolate is completely inhibitory at 1 ppm. Assuming that complete dissociation occurred, about 0.8 ppm of 8-hydroxyquinoline and 0.2 ppm of copper could have been formed. However, it is shown that as much as 1 ppm of 8-hydroxyquinoline shows no inhibition and none can be attributed to the copper alone at even higher concentrations. Thus, the copper must be performing an important role in cooperation with the function of the 8-hydroxyquinoline.

Further support of the statement that the copper and 8-hydroxyquinoline function synergistically was obtained when 1 ppm of 8-hydroxyquinoline and 5 ppm of copper sulfate were added to the medium. These concentrations did not produce any inhibition when the compounds were tested separately, but when combined, resulted in 100 percent inhibition.

DISCUSSION

It is particularly significant that the reversals of the activity of 8-hydroxyquinoline and copper 8-quinolinolate required a considerable excess of metal ions over the stoichiometric amount. Zentmeyer (**13**), using about three times the stoichiometric amount of zinc, achieved but a 78 percent reversal. This indicates that the reversal of the activity by metal ions is being opposed; dissociation of the metal quinolinolate is the most plausible cause of the opposition. Just on the basis of the reversal of the activity of 8-hydroxyquinoline by metal ions, it would appear that a metal quinolinolate would not exhibit any activity. This,

of course, is not true and the equilibrium equation previously discussed resolves this difficulty.

The factors which account for the dissociation of the copper 8-quinolinolate may be several. It is conceivable that the fungus could hydrolyze the compound. Ionization in water is another potential cause of dissociation. While the compound is very insoluble, the data show that it is completely inhibitory in the agar toxicity test when used at concentrations approximating the saturation level.

The theory implies that the fungus may have at its command some means of inactivating 8-hydroxyquinoline; this follows from the fact that the compound is much less effective than its copper salt. It is quite possible that a mold enzyme, a phenol oxidase for example, could oxidize the 8-hydroxyquinoline to a compound which would not precipitate the essential metal ions and accordingly would show no activity. Some evidence in support of this conjecture was obtained in tests which showed that a lignin oxidase, isolated from fungi, oxidized 8-hydroxyquinoline. This work was done by Gottlieb and Geller according to procedures they have developed (**5**).

The theory clearly indicates that, in the case of copper 8-quinolinolate, the fungistatic activity cannot be attributed to the copper alone. Many reports lead one to believe that copper organic compounds owe their activity to the copper and that the remainder of the molecule merely determines how firmly the copper will be bound to the textile or other material to which it is applied. These findings and others (**9**) clearly indicate that this hypothesis can no longer be seriously entertained as a general rule.

PRACTICAL IMPLICATIONS

The theory opens several approaches for the development of new fungicides. First, since copper 8-quinolinolate does not function as a molecular entity, it is not necessary to use the compound as such to achieve its desirable fungistatic effects; any copper salt can be mixed with 8-hydroxyquinoline, or certain of its derivatives, to produce fungistatic activity equivalent to that of the copper 8-quinolinolate. Several such mixtures were incorporated into a paint which was then applied to filter paper for testing. The painted paper was placed on a mineral salts-nutrient-agar medium, inoculated, and incubated. Details of this procedure have been reported by the two senior authors (**12**). A representative group of results is summarized in TABLE II. It is shown that at concentrations where the 8-hydroxyquinoline and copper salt, such as the carbonate, are completely ineffective when tested separately, the com-

bination of the two completely prevents the growth of the test organism. With reference to the theory, these results again show that copper 8-quinolinolate is much more effective than 8-hydroxyquinoline and that there is a striking synergism between the copper ions and 8-hydroxyquinoline.

It is apparent, also, that through the use of various copper salts and 8-hydroxyquinoline derivatives, numerous mixtures with different physical and chemical properties can be prepared, thus providing desirable properties for various applications. For example, copper 8-quinolinolate cannot be used in white paints because of its green-yellow color; however, 8-hydroxyquinoline can be mixed with a light colored copper salt to produce a highly effective mixture with little or no effect on the

TABLE II

FUNGICIDAL ACTIVITY OF 8-HYDROXYQUINOLINE MIXED INTO PAINT WITH SEVERAL COPPER COMPOUNDS

Compound	Conc. % by wt.	*Aspergillus oryzae*	*Aspergillus niger*
8-hydroxyquinoline	1.0	3*	3
	2.0	3	3
	4.0	2	2
Copper 8-quinolinolate	0.5	1	1
	1.0	1	1
8-hydroxyquinoline plus copper carbonate	0.5**	1	1
	1.0**	1	1
Copper carbonate	0.5	4	2
8-hydroxyquinoline plus copper 3-phenylsalicylate	0.5**	1	1
	1.0**	1	1
Copper 3-phenyl-salicylate	0.5	3	3
	1.0	3	3

* Numbers indicate amount of mold growth appearing on the test specimen as follows: 1 = no growth; 2 = slight growth; 3 = moderate growth; 4 = heavy growth.

** The concentration given is the percentage of each compound mixed into the paint.

color of the paint. In another case, it has been found that copper 8-quinolinolate is not compatible with certain vinyl plastic films and slowly migrates to the surface of the film. By proper selection of a copper salt and an 8-hydroxyquinoline derivative, this difficulty was overcome without sacrifice of fungistatic activity. Also under investigation is the determination of the optimum copper-hydroxyquinoline ratio of these mixtures. It is probable that the copper which combines stoichiometrically in the formation of copper 8-quinolinolate does not represent the optimum ratio for achieving maximum activity.

A second approach to the development of new fungicides is to use chelating agents other than 8-hydroxyquinoline. Some work on other che-

lating agents, particularly as bactericides (**1**), has been reported. The authors have synthesized and evaluated numerous chelating agents and will report these findings later. A large proportion of the chelating agents show activity, thereby contributing further support to the idea that chelation is a factor in their mechanism of action.

The analytical reagent, the ammonium salt of N-nitroso-N-phenylhydroxylamine, is one of the more promising fungicides. Zentmeyer (**13**) reported that it was fungistatic and Illman *et al.* (**7**) recommended the use of its copper salt in preference to copper 8-quinolinolate for the treatment of textiles. The authors prepared and tested this copper salt in paint and textiles; in textiles it approximated the activity of copper 8-quinolinolate, but in paint it was somewhat less effective. The color of the compound, gray, is advantageous.

It is significant to note that in the case of the ammonium salt of N-nitroso-N-phenylhydroxylamine, combination with copper does not produce a synergism as striking as in the case of 8-hydroxyquinoline. However, the copper salt is used because of the instability of the ammonium salt itself. The copper salt is very insoluble, as is copper 8-quinolinolate, and special techniques are required to apply it to various materials.

ACKNOWLEDGMENTS

The authors wish to thank S. Gottlieb for conducting the tests on the enzymatic oxidation of 8-hydroxyquinoline and P. J. Hannan and Mrs. J. V. Gartlan for their assistance in conducting the biological tests and preparing the compounds.

A part of this work was submitted to Georgetown University by M. Manowitz in fulfillment of his thesis requirement.

SUMMARY

A theory of the mechanism of fungistatic action of copper 8-quinolinolate is proposed. It is suggested that molecules of the copper salt must first dissociate to form copper ions and 8-hydroxyquinoline, each of which performs different but uniquely related functions. The 8-hydroxyquinoline inhibits the growth of fungi by precipitating those metals which are essential for their metabolism (**1, 13**). The copper ions function synergistically with the 8-hydroxyquinoline. In addition to discussing the theory, some of its practical implications are presented.

ENGINEER RESEARCH AND DEVELOPMENT LABORATORIES
THE ENGINEER CENTER AND FORT BELVOIR
FORT BELVOIR, VIRGINIA

LITERATURE CITED

1. **Albert, A., S. D. Rubbo, R. Goldacre and B. Balfour.** 1947. The influence of antibacterial activity. Part III: A study of 8-hydroxyquinoline (Oxine) and related compounds. Brit. Jour. Exptl. Path. **28**: 69–87.
2. **Benignus, P. G.** 1948. Copper 8-quinolinolate industrial preservative. Ind. Eng. Chem. **40**: 1426–1429.
3. **Eisenschiml, O. and V. N. Kalberg.** 1948. Fungicides in protective coatings. Paint, Oil & Chem. Rev. **111**: 17–19; 29–30.
4. **Flagg, J. F.** 1948. Organic reagents. Interscience Publishers, New York.
5. **Gottlieb, S. and J. H. Geller.** 1949. Enzymatic decomposition of lignin. Science **110**: 189–190.
6. **Hatfield, I., P. G. Benignus and D. F. Rogers.** 1944. 8-hydroxyquinoline and its salts as textile preservatives. Monsanto Chemical Co., St. Louis, Mo.
7. **Illman, W. I., G. Semenuik, A. C. Neish and G. A. Ledingham.** 1948. Rot resistance of cotton duck treated with chelate copper compounds. Can. Jour. Research F, **26**: 311–317.
8. **Maley, L. E. and D. P. Mellor.** 1949. The relative stability of internal metal complexes. I. Complexes of 8-hydroxyquinoline, salicylaldehyde, and acetylacetone. Australian Jour. Sci. Research A, **2**: 92–110.
9. **Marsh, P. B., G. A. Greathouse, K. Bollenbacher and M. L. Butler.** 1944. Copper soaps as rot-proofing agents on fabrics. Ind. Eng. Chem. **36**: 176–181.
10. **Rigler, N. E. and G. A. Greathouse.** 1941. Fungicidal potency of quinoline homologs and derivatives against *Phymatotrichum omnivorum*. Ind. Eng. Chem. **33**: 693–694.
11. **Rubbo, S. D.** 1949. The mode of action of 8-hydroxyquinoline (Oxine); a biological approach. Intern. Congr. Microbiol., Rept. Proc. 4th Congr., Copenhagen, 1947: 149–150.
12. **Vicklund, R. E. and M. Manowitz.** 1947. Test methods for evaluating paint fungicides. Am. Paint Jour. **32**: 67–68; 70; 72; 75; 78; 80; 82; 86–89.
13. **Zentmeyer, G. A.** 1944. Inhibition of metal catalysis as a fungistatic mechanism. Science **100**: 294–295.

THE SENSITIZATION OF ASCOSPORES TO CHEMICAL ACTIVATORS BY HEAT TREATMENT[1, 2]

ALFRED S. SUSSMAN

(WITH 4 FIGURES)

Although freshly harvested ascospores of *Neurospora* germinate in response to catalytic amounts of furfural and certain related chemicals, ascospores which have aged become almost refractory to such treatment (Emerson, **1951**; Sussman, **1953b**). The following experiments will show that spores which no longer respond to chemical activators can regain their sensitivity to such treatment by means of short exposures to temperatures between 40–50° C.

MATERIALS AND METHODS

The ascospores of *Neurospora tetrasperma* used in these experiments were obtained as described by Goddard (**1935**). They were stored over 65% humidity for varying periods of time until use. Experiments at constant temperature were performed by means of the magnetic-stirring assembly described by Lowry and Sussman (**1953**). The volume of a suspension of ascospores in distilled water was determined by means of a hematocrit tube. 200 times this volume of distilled water (usually between 40–50 ml) was added to a bottle having a serum port and an arm permitting the insertion of a thermometer. The bottle and its contents were then equilibrated to the temperature of the oil bath. At zero time, ascospores prepared as above, and suspended in 1 ml of distilled water, were introduced into the bottle by means of a 2 ml syringe. Under these conditions, equilibration of the spores to the constant-temperature oil bath was almost immediate. 1 ml aliquots of the ascospores were extracted at intervals thereafter and dispensed into 25 ml Erlenmeyer flasks containing 1 ml of activating chemicals or water. Incubation at 25° C was accomplished on a reciprocal shaking machine at 200 rpm.

[1] The author would like to express his appreciation to the Committee on Growth of the American Cancer Society and to the Rackham Fund of the University of Michigan for supporting these investigations.

[2] Papers from the Department of Botany of the Unversity of Michigan, No. 992.

At the end of 6 hours the spores were formalinized and counted as described previously (Sussman, **1953a**).

Respirometric experiments were performed with the Warburg apparatus using 7 ml vessels and a total fluid volume of 1.1 ml. All such experiments were carried out at 26° C.

The furfural, pyrrole, and ethyl ether used were vacuum distilled before use; the other chemicals used were either the commercial product or were furnished by the research laboratories of the following organizations: The Monsanto Chemical Co., Inc.; The Eaton Laboratories, Inc.; Electrochemicals Department, DuPont de Nemours and Co.

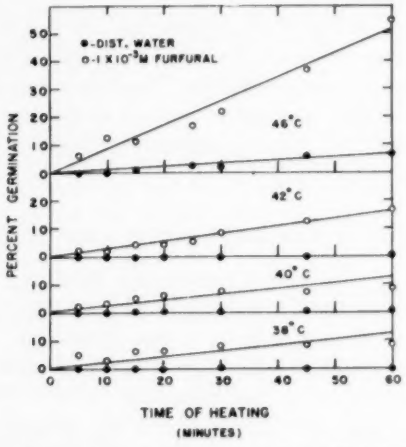


FIG. 1. The effect of exposure to temperatures from 38–46° C upon the response of ascospores of *Neurospora tetrasperma* to furfural (1×10^{-3}M). Ascospores were used 28 months after harvesting.

RESULTS

Ascospores which had been harvested approximately 28 months previously ("old" spores) were heated at several temperatures for various periods of time. The percentage germination of these spores in distilled water and in 1×10^{-3}M furfural was determined as described previously. FIGS. 1 and 2 show that heating ascospores for one hour or less at temperatures ranging from 38° C–50° C sensitizes them to furfural. At 46° C and below, little heat activation of the ascospores results after heating up to one hour. However, at 48° C and above, considerable such activation occurs within this time interval so that it is difficult to distinguish between this effect and the effect of furfural.

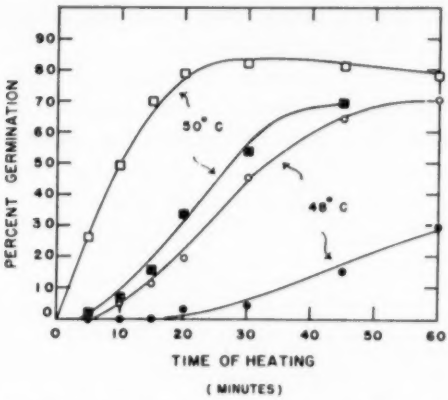


FIG. 2. The effect of exposure to temperatures from 48–50° C upon the response of ascospores of *Neurospora tetrasperma* to furfural (1×10^{-3}M). Ascospores were used 28 months after harvesting. The empty squares and circles plot the values for the furfural-treated samples while the filled squares and circles plot those for the water controls.

One-month-old ascospores ("fresh" spores) were then heated to 44° C for the same time periods as were used in the preceding experiments. The results are given in TABLE I and it can be seen that although these "fresh" spores are still sensitive to furfural, the percentage germination in the presence of this chemical is almost tripled as a result of heating for one hour.

The effect of heat upon the sensitivity of ascospores to chemical activators other than furfural was then investigated. Spores which had been harvested 75 days previously were heated at 46° C for 30 minutes after which 1 ml aliquots were added to solutions of several of the chemical activators listed by Sussman (**1953a**). The results in TABLE II show

TABLE I

THE EFFECT OF HEATING AT 44° C UPON THE RESPONSE OF ASCOSPORES OF NEUROSPORA TETRASPERMA TO FURFURAL. THE ASCOSPORES WERE HARVESTED 30 DAYS BEFORE USE

	Percentage germination in:	
Time of heating (minutes)	H_2O	1×10^{-3}M furfural
0	0	25
5	0	32
10	0	32
15	0	42
20	0	45
30	0	44
45	0	59
60	0	71

that the sensitivity of ascospores to these chemicals, as well as to furfural, is markedly increased by heat treatment.

Experiments were then performed to determine how long heat-treated ascospores retained their sensitivity to activators. With this in mind, ascospores harvested 28 months previously were heated to 46° C for 30 minutes and immediately cooled. A 1 ml aliquot (0-hour sample) was extracted and added to enough furfural to give a final concentration of 1×10^{-3}M. These spores were incubated at 26° C for 6 hours after

TABLE II

THE RESPONSE OF ASCOSPORES OF NEUROSPORA TETRASPERMA TO CHEMICAL ACTIVATORS AFTER HEAT-TREATMENT AT 46° C FOR 30 MINUTES. THE ASCOSPORES WERE USED 75 DAYS AFTER BEING HARVESTED

Compound	Concentration	Percent germination	
		Unheated spores	Heated spores
Pyrrole	1×10^{-2}M	7.4	55
	1×10^{-3}M	2.2	26
2-methyl furan	1×10^{-2}M	10.5*	64
	1×10^{-3}M	0.8*	4
2-furfural diacetate	1×10^{-3}M	9.6	63
2-bromothiophene	1×10^{-3}M	5.0	8
Thiophene	1×10^{-2}M	3.0	8
	1×10^{-3}M	1.8	3
2-thiophene carboxyaldehyde	1×10^{-2}M	2.0	2
	1×10^{-3}M	5.7	66
Diethyl ether	1×10^{-1}M	4.8	18
2-furfural	1×10^{-2}M	10.9*	47*
	1×10^{-3}M	31	78
Distilled water	—	0.8	3

* Germination never progressed beyond the germ tube stage.

which they were formalinized and counts made as before. The remainder of the ascospores, suspended in about 50 ml of distilled water in a 125 ml Erlenmeyer flask, were shaken in a rotary shaker at 26° C and 1 ml aliquots were extracted periodically. Such aliquots were added to furfural, and were incubated, formalinized, and counted as described for the 0-hour sample. The results are given in FIG. 3 and show that heated ascospores lose their sensitivity to furfural within 4 hours after shaking at 26° C. About ½ their sensitivity is lost within 45 minutes after shaking is begun.

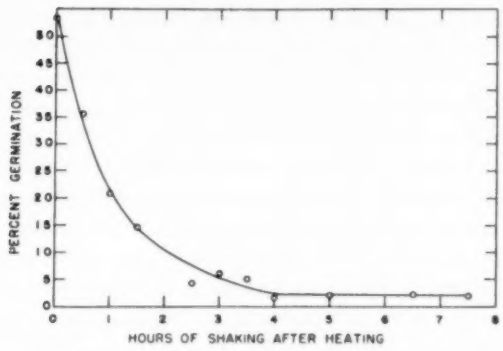


FIG. 3. The loss of sensitivity of ascospores of *Neurospora tetrasperma* to chemical activators as a result of aerobic shaking.

In order to determine whether the amount of aeration influenced the loss of sensitivity, a sample of the same lot of ascospores was heated as before and was divided into 2 lots. The first lot was shaken in a rotary shaker at 26° C and 1 ml aliquots were extracted periodically and were incubated in 1×10^{-3}M furfural. The second lot of ascospores was divided between 4 Thunberg tubes from which the air was immediately evacuated by means of a Cenco Hyvac pump. These tubes were also incubated at 26° C and were opened at different times to permit the extraction of 1 ml samples which were then treated as described above. TABLE III shows that under conditions where oxygen supply is restricted in this way ascospores lose their sensitivity to furfural more slowly than when aeration is vigorous.

Since the activation process in *Neurospora* ascospores is accompanied by characteristic respiratory changes (Goddard, **1939**; Sussman, **1953b**), such changes were investigated in spores whose response to furfural had been altered by heat-treatment. "Old" spores (harvested 28 months

TABLE III

THE EFFECT OF ANAEROBIOSIS UPON THE LOSS OF SENSITIVITY TO FURFURAL OF ASCOSPORES OF NEUROSPORA TETRASPERMA WHICH WERE HEATED TO 46° C FOR 30 MINUTES

Time of incubation after heat treatment (hrs.)	Percent germination	
	Conditions of incubation	
	Aerobic	Anaerobic
0	45	—
1	21	39
2	5	20
4.5	2	14
23	2	10

previously) were suspended in distilled water and aliquots were pipetted directly into Warburg vessels. The remainder of the spores were heated for 30 minutes at 46° C and immediately cooled and distributed into other Warburg vessels. The oxygen uptake of these two groups of spores was determined in distilled water and in 1×10^{-3}M furfural and the results plotted in FIG. 4. These results indicate that there is a 3-fold increase in the oxygen consumption of ascospores which have been sensitized to furfural by heat treatment at 46° C for 30 minutes.

In order to determine whether the increase noted above was due solely to the increase to be expected on the basis of a temperature coefficient (Q_{10}) of 2–3, "fresh" spores were exposed to a temperature of

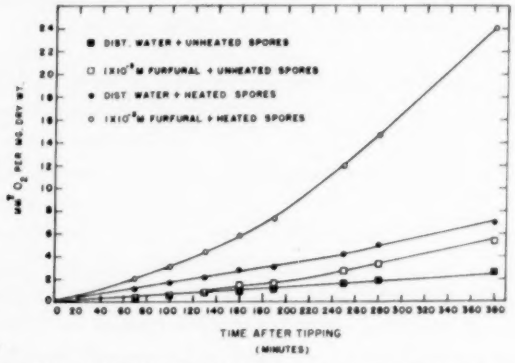

DIST. WATER + UNHEATED SPORES
1X10⁻³M FURFURAL + UNHEATED SPORES
DIST. WATER + HEATED SPORES
1X10⁻³M FURFURAL + HEATED SPORES
MM³ O₂ PER MG. DRY WT.
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380
TIME AFTER TIPPING (MINUTES)

FIG. 4. The effect of heating at 46° C for 30 minutes upon the oxygen uptake of "old" ascospores of *Neurospora tetrasperma*. The furfural was tipped at zero time.

46° C for only 5 minutes and the respirometric experiments described above were repeated. The results given in TABLE IV indicate that spores treated in this manner showed a 2-fold rise in oxygen uptake. Under these conditions little sensitization to furfural occurred.

DISCUSSION AND CONCLUSIONS

The change in ascospores of *Neurospora* which results in their loss of sensitivity to chemical activators has been shown to be reversed by short exposures to temperatures between 38–50° C. Such treatment is effective in sensitizing the ascospores to several chemical activators, including furfural. Above 46° C, heat activation obscures this effect although it is still recognizable at 50° C.

Under conditions of maximum aeration after heat treatment, ascospores lose their sensitivity to chemical activators within 4 hours after such treatment. On the other hand, under a reduced supply of oxygen, about 20% of the spore's sensitivity is retained for at least 23 hours.

The shape of the curve for loss in sensitivity with aeration (FIG. 3) is that of a first-order chemical reaction. That this reaction may be enzymatic and concerned with the cell's metabolism is suggested by the fact that it proceeds faster when oxygen is furnished.

Heat-sensitization of ascospores appears to be accompanied by a 2–3-fold increase in oxygen uptake. That such increases are obtained when ascospores are exposed to 46° C for only 5 minutes suggests that an adjustment to higher temperature such as can be expected on the basis of a temperature coefficient (Q_{10}) of 2–3 cannot explain this effect. Since the rate of oxygen uptake is linear for at least 4 hours after measurements are begun, it is felt that equilibration to the temperature of

TABLE IV

THE EFFECT OF HEATING UPON THE OXYGEN UPTAKE OF "FRESH" ASCOSPORES OF NEUROSPORA TETRASPERMA. THE ASCOSPORES WERE HEATED AT 46° C FOR 5 MINUTES

	Q_{O_2}*	
	Unheated spores	Heated spores
Distilled water	0.2	0.45
1×10^{-3}M furfural	4.2	6.0

* The Q_{O_2} was calculated as the mm^3 O_2/mg dry wt./hr. during the time interval 240–300 minutes after measurements were begun.

the Warburg bath must be very rapid. Although this rise in oxygen uptake seems to be real there is some doubt as to whether it is responsible for the increased sensitivity of the ascospores to chemical activators. For example, despite the complete loss in sensitivity after aerobic incubation for 4 hours, the respiratory rate remains linear for this period of time. If there were any direct relation between the two phenomena it would be expected that the respiratory rate would parallel the loss in sensitivity to activators. Furthermore, it would also be expected that freshly harvested ascospores which are quite sensitive to furfural would have a higher rate of oxygen uptake than that of older ones. However, the data in FIG. 4 and TABLE IV show the reverse to be true, since the Q_{O_2} of "old" ascospores is 0.4 compared with that of the "fresh" lot which is 0.22. Finally, although exposure to 46° C for 5 minutes did not sensitize the spores appreciably, a 2-fold increase in the Q_{O_2} occurred. Therefore, the respiratory increase, taken by itself, cannot explain the sensitization of ascospores to chemical activators.

SUMMARY

Short exposures of ascospores of *Neurospora tetrasperma* to temperatures between 38°–50° C have been shown to increase the activating effect of furfural. 28-month-old ascospores, as well as more recently harvested ones, respond to such treatment. The activating effect of chemical activators other than furfural has also been shown to be enhanced by heat-treatment. Aerobic incubation of heat-treated ascospores causes a rapid loss in sensitivity to furfural. On the other hand, such sensitivity is retained for longer periods of time under conditions where oxygen is limiting. A 2–3-fold increase in respiratory rate accompanies heat-sensitization but does not seem to be the cause of such sensitization.

DEPARTMENT OF BOTANY,
UNIVERSITY OF MICHIGAN, ANN ARBOR

REFERENCES CITED

Emerson, Mary. 1951. Personal communication.

Goddard, D. R. 1935. The reversible heat activation inducing germination and increased respiration in the ascospores of *Neurospora tetrasperma*. Jour. Gen. Physiol. **19**: 45–60.

——. 1939. The reversible heat activation of respiration in *Neurospora*. Cold Spr. Harb. Symp. Quant. Biol. **7**: 362–376.

Lowry, R. J. and A. S. Sussman. 1953. A magnetic stirring device for use in constant-temperature studies with microorganisms. In press.

Sussman, A. S. 1953a. The effect of heterocyclic and other compounds upon the germination of ascospores of *Neurospora tetrasperma*. Jour. Gen. Microbiol. **8**: 211–216.

——. 1953b. The effect of furfural upon the germination and respiration of ascospores of *Neurospora tetrasperma*. Amer. Jour. Bot. **40**: 401–404.

SPORE GERMINATION IN USTILAGO ZEAE AS INFLUENCED BY KH_2PO_4 [1]

THOR KOMMEDAHL

Experiments were made to determine the effect, if any, of radiophosphorus in KH_2PO_4 upon the germination of chlamydospores from *Ustilago zeae* (Beckm.) Ung. However, it was not known whether KH_2PO_4, as such, might affect spore germination, although the effects of some other substances are known. For example, Leszczenko (**3**) found that the following acids and their potassium salts at low concentrations stimulated germination: acetic, citric, muriatic, nitric, orthophosphoric, oxalic, and sulfuric. He also found that such alkalies as sodium, potassium, and ammonium hydroxide stimulated germination of chlamydospores from *U. zeae*, when compared with germination in distilled water.

Effects of radiation on germination of *U. zeae* chlamydospores have been reported. Schmitt (**8**) observed a delay in germination of irradiated spores and that the delay increased with the dosage applied, when monochromatic ultraviolet radiation was applied to the 5th inbred generation chlamydospores. Also, Pichler and Wöber (**5**) noted that with *U. zeae*, exposure of dry spores to ultraviolet rays inhibited their germination.

MATERIALS AND METHODS

The chlamydospores of *U. zeae* were obtained from a single corn smut gall, and germination studies were made within 9 months of collection. Czapek's sucrose-nitrate agar was used throughout, since KH_2PO_4 is a constituent of this medium at an amount of 1 g/l. Radiophosphorus, a beta emitter, was used in the form of $KH_2P^{32}O_4$.

Dry chlamydospores were brushed aseptically upon an agar surface using a camel's hair brush. After brushing, the Petri plates were placed in a refrigerator (3° C) and left there for time-periods that varied from 2 to 30 days. At the end of each time-period, the plates were transferred to an incubator (27° C). This incubation temperature was opti-

[1] Work was done under Contract No. AT (11-1)-29 for the Atomic Energy Commission. Technical assistance of Nancy J. Hockman is acknowledged. Journal Article No. 26-53 of the O. A. E. S.

mum according to Jones (2), Piemeisel (6), and Schmitt (8). Twenty-four to 28 hours later, plates were removed from the incubator and counts were made of the number of germinated spores in the first 500 spores encountered per agar block.

Because standard Czapek's agar contains 1 g of KH_2PO_4 per liter of medium, this served as a check. At this level, it was possible to obtain initial specific activities of 0.025, 0.05, 0.1, and 0.2 microcuries (μc) per ml of medium, using different proportions of radioactive and non-radioactive KH_2PO_4. To attain initial specific activities of 5, 10, 20, and 40 μc of P^{32} per ml, it was necessary to add 4, 8, 16, and 32 g, respec-

TABLE I

THE PERCENTAGE GERMINATION OF CHLAMYDOSPORES FROM USTILAGO ZEAE AFTER INCUBATION AT 27° C FOR 24–28 HOURS FOLLOWING 30-DAY STORAGE AT 3° C ON CZAPEK'S AGAR IN WHICH KH_2PO_4 WAS VARIED IN CONTENT AND IN RADIOACTIVITY

KH_2PO_4 in grams/liter	Initial specific activity of P^{32} in μc/ml	No. spores counted	Percentage spores germinated
1	0.	7365	73
	0.025	2635	72
	0.05	2599	72
	0.1	2663	75
	0.2	4076	74
4	0.	2660	68
	5.	2433	67
8	0.	2598	70
	10.	2666	60
16	0.	2775	50
	20.	2804	49
32	0.	2806	44
	40.	2568	36

tively, of $KH_2P^{32}O_4$ per liter of Czapek's agar. As a check on the effect of P^{32}, 4, 8, 16, and 32 g of $KH_2P^{31}O_4$ were added to a second set of media.

EXPERIMENTAL RESULTS

Effects on germination of 30-days' exposure to KH_2PO_4. From the data in TABLE I, it is evident that the striking effect from high concentrations of KH_2PO_4 in agar was due to the compound as such, and not to its radioactivity. It was found that 73 percent of the chlamydospores from *U. zeae* germinated on standard Czapek's medium (1 g/l of

KH_2PO_4). However, at the highest level of KH_2PO_4, germination had decreased to 44 percent—a decrease in germination of 29 percent. Also, at the next to the highest level, there was a marked reduction from the check in germination of chlamydospores, from 73 to 50 percent. However, where the KH_2PO_4-content varied from 4 to 8 grams per liter of medium, the percentage germination of spores did not differ appreciably from that which occurred on the standard medium. It is apparent therefore that with an increase in the amount of KH_2PO_4 in Czapek's agar, there is a striking decrease in the numbers of spores that germinate. Furthermore, from TABLE I it is evident that radioactivity of the medium is not influential in altering materially the germination of chlamydospores.

Hour of germination. Experiments were set up to determine the effect of KH_2PO_4 on the numbers of spores germinating at a given hour of incubation. Hourly, from the twentieth to the thirtieth hour, 500 to 700 spores were counted, and the number germinated was determined. Prior to incubation, spores-on-agar were refrigerated for 30 days to allow time for spores to be exposed to P^{32} and yet not germinate. Chlamydospores were exposed to the 4 levels of radiation and the corresponding level of KH_2PO_4.

It was discovered that the highest incidence of germinated spores occurred on media where the standard recipe for Czapek's agar was followed. Furthermore, neither 4 nor 8 g/l, of KH_2PO_4, altered germination appreciably from that of the standard medium. However, at the two highest levels, there was a marked reduction in the percentage of spores that germinated per hour, from the twentieth to the thirtieth hour. It was noteworthy for example that at the twentieth hour of incubation, 64 percent of the spores of the check (1 g/l) germinated while at the highest level (32 g/l) 38 percent germinated—a decrease of 26 percent. Similarly at the thirtieth hour, 74 percent of the spores germinated upon the check medium, while at the highest level of KH_2PO_4, 49 percent of the spores germinated, a decrease of 25 percent—virtually the same difference as that at the twentieth hour of incubation. Approximately the same differences in germination occurred for the intervening hours of incubation. There was a gradual increase in germination from the twentieth to the thirtieth hours at each level of KH_2PO_4. The reduction in germination with 4 and 8 g of KH_2PO_4 per liter of medium was small when compared to the 1 g/l level; however at the 16 g level, the percentage germination varied from 42 at the twentieth hour to 62 at the thirtieth.

In conclusion, there was a considerable reduction in spore germination

when the KH_2PO_4 content of Czapek's medium was increased from 16 to 32 times that of the standard recipe. However, radiophosphorus at the 4 levels used had no effect on numbers of spores that germinated.

Day of germination. Each day for 10 days and on alternate days for the succeeding 20, agar blocks were transferred from the refrigerator to an incubator, and after 24 hours of incubation, the numbers of germinated spores were determined. During this 30-day period, the percentage germination on standard agar varied from 60 to 72, with a mean percentage of 65, based upon 3000 to 4000 spores counted per day. There was no tendency for germination to increase nor decrease after 1 to 30 days of refrigeration. Of 4200 spores on standard agar incubated without previous refrigeration, 53 percent germinated in 24 hours —not significantly different from refrigerated spores.

When the KH_2PO_4 content was increased to 4, 8, or 16 grams per liter of medium, the percentage germination remained in the sixties on each of the days of the 30-day period when counts were made, based on 500 to 600 spores counted per day. However at the highest level of KH_2PO_4, there was a lag in germination up to the eleventh day when the incidence of germinated spores approached that on the standard medium. On the eighteenth day of refrigeration, spore germination decreased from 68 to 53 percent, and after 30 days, from 65 to 44 percent. When P^{32} was substituted for P^{31}, there was little difference in germination.

In general, with KH_2PO_4 at 1, 4, 8, and 16 grams per liter of medium, the percentage germination remained unchanged with time of refrigeration from the second to the thirtieth day. However at the highest level (32 g/l), there was a marked reduction in germination after the eighteenth day of storage at 3° C. P^{32} up to 40 μc/ml of medium failed to influence in any way the incidence of spores that germinated.

DISCUSSION

It was obvious from the foregoing experiments that P^{32} in KH_2PO_4 could not be used as a source of radiation because it was impossible to attain high enough specific activities to get an effect from radiation without first getting an effect from the increased amount of KH_2PO_4 as such. However, from the work of others, spore germination in general is apparently not affected by low radiations of limited duration. For example, Pearson *et al.* (**4**) found no effect from exposure of fungus spores for 48 hours to 20 μc of P^{32}. Wheeler (**10**) reports similar results. Waggoner and Dimond (**9**) found that there was no reduction in germination when spores from *Fusarium oxysporum* f. *lycopersici* were

exposed to gamma radiation of 910 r, and that at 31,500 r there was only an 11 percent reduction.

Since there were virtually no differences in pH among the variations in formulations of Czapek's medium used, the results are not attributable to any such effect. Moreover, Itzerott (**1**) found that chlamydospores of *U. zeae* germinated best at pH 4.4, decreasing until arrested at pH 2.5, while germination of young spores was not impaired from pH 4.4 to 8.55.

Although variations in germination are frequently reported, it is possible that brushing spores upon an agar surface imbeds them unequally into the medium, favoring germination of some more than others, thus accounting partially for the variation. That this might be so is illustrated by Platz (**7**) who reported that chlamydospores of *U. zeae* failed to germinate when completely immersed in media and kept in air-tight vials; neither did germination occur in less than 3 percent oxygen.

SUMMARY

When the KH_2PO_4 content of Czapek's medium was exceeded by from 16 to 32 times the amount called for in the recipe (1 g/l), chlamydospore germination in *Ustilago zeae* was reduced by as much as 30 percent—from 73 percent spore germination on standard Czapek's agar to as low as 36 to 44 percent germination at 32 g KH_2PO_4 per liter of Czapek's medium. The effect in the medium of KH_2PO_4 upon the germination of spores was most pronounced when spores were exposed to this medium for 20 to 30 days at 3° C, followed by incubation at 27° C for 24 to 28 hours.

It was not possible to use P^{32} in KH_2PO_4 as a source of radiation in order to study the effect on germination of chlamydospores of *U. zeae*, because it was necessary to add so much KH_2PO_4 to the medium in attaining high initial specific activities that there was an effect from KH_2PO_4 as such, upon the germination of spores. Beta radiation from radiophosphorus in KH_2PO_4 at the initial specific activities of 0.025, 0.05, 0.1, 0.2, 5, 10, 20, and 40 μc/ml failed to influence the number of chlamydospores of *U. zeae* that germinated.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY,
OHIO AGRICULTURAL EXPERIMENT STATION,
WOOSTER, OHIO

LITERATURE CITED

1. **Itzerott, Dorothea.** 1938. Über Keimung und Wachstum von *Ustilago zeae* (Beckm.) Ung. mit besonderer Berücksichtigung der Infektion. Phytopath. Zeitschr. **11**: 155–180.

2. **Jones, Edith S.** 1923. Influence of temperature on the spore germination of *Ustilago zeae*. Jour. Agric. Res. **24**: 593–597.
3. **Leszczenko, P.** 1926. On the influence of reaction of the medium on the germination of the spores of *Verticillium albo-atrum* R. & B. and *Ustilago maydis* Tul. Mem. Inst. nat. Polonais d'Econ. Rur. a Putawy **7(A)**: 402–435. (Abs. Rev. App. Myc. **6**: 307. 1927.)
4. **Pearson, I. A., J. M. Hammer, K. E. Corrigan and H. S. Hayden.** 1948. Studies on the behavior of fungi in the presence of radioactive isotopes. Jour. Bact. **56**: 397–402.
5. **Pichler, F. and A. Wöber.** 1922. Bestrahlungsversuche mit ultra-violettem Licht, Röntgenstrahlen und Radium zur Bekämpfung von Pflanzenkrankeiten. Centralbl. fur Bakt. Abt. *2*, **57**: 319–327.
6. **Piemeisel, F. J.** 1917. Factors affecting the parasitism of *Ustilago zeae*. Phytopathology **7**: 294–307.
7. **Platz, G. A.** 1928. The relation of oxygen to the germination of the chlamydospores of *Ustilago zeae* (Beckm.) Ung. Iowa State Coll. Jour. Sci. **2**: 137–143.
8. **Schmitt, G. G.** 1940. Cultural and genetic studies on *Ustilago zeae*. Phytopathology **30**: 381–390.
9. **Waggoner, P. E. and A. E. Dimond.** 1952. Examination of the possibility of therapy of plant disease with ionizing radiation. Phytopathology **42**: 599–602.
10. **Wheeler, H. E.** 1952. The use of radiocarbon for tagging fungi. Phytopathology **42**: 431–435.

THE PARASITIC GROWTH PHASE OF COCCIDIOIDES IMMITIS IN CULTURE

N. F. Conant and R. A. Vogel[1]

(with 4 figures)

The following note adds to a growing list of reports that the parasitic growth phase of *Coccidioides immitis* is frequently seen in mycelial cultures (**1**, **2**, **4**), or can be induced to form consistently on synthetic media (**3**).

EXPERIMENTAL

During the course of recent work on the cultivation of *C. immitis* in the embryonated hen's egg (**8**) it was found that only old cultures with prolific arthrospore formation gave best conversions and quantity of spherule material in the yolk sac of the inoculated eggs. All cultures had to be examined microscopically, therefore, to be assured of the presence of abundant sporulation. Also, frequent transfers of such old powdery friable cultures were necessary to maintain stock cultures during the experiment.

To protect the operator during such potentially dangerous procedures (**5**, **7**) the growth on slants was first wet down with sterile saline containing 0.05–0.1% "Tween 80." The growth was gently loosened from the agar surface with a wire loop and, after removal of a drop of the suspension for microscopic examination, the remainder was pipetted into a sterile vaccine bottle for egg inoculation and onto several Sabouraud's dextrose agar slants to perpetuate stock cultures.

RESULTS

Subcultures from Tween-treated inoculum developed a moist mycelial mat on the agar surface with little tendency to produce aerial growth. Microscopic examination of two strains after four months' growth revealed no arthrospores in strain *973* and very few in strain *2082*. Other strains also showed varying degrees of reduced sporulation.

In place of the usual arthrospore formation originally seen in strains *973* and *2082* were found numerous enlarged rounded chlamydospore-

[1] Present address: Veterans Hospital, Atlanta, Georgia.

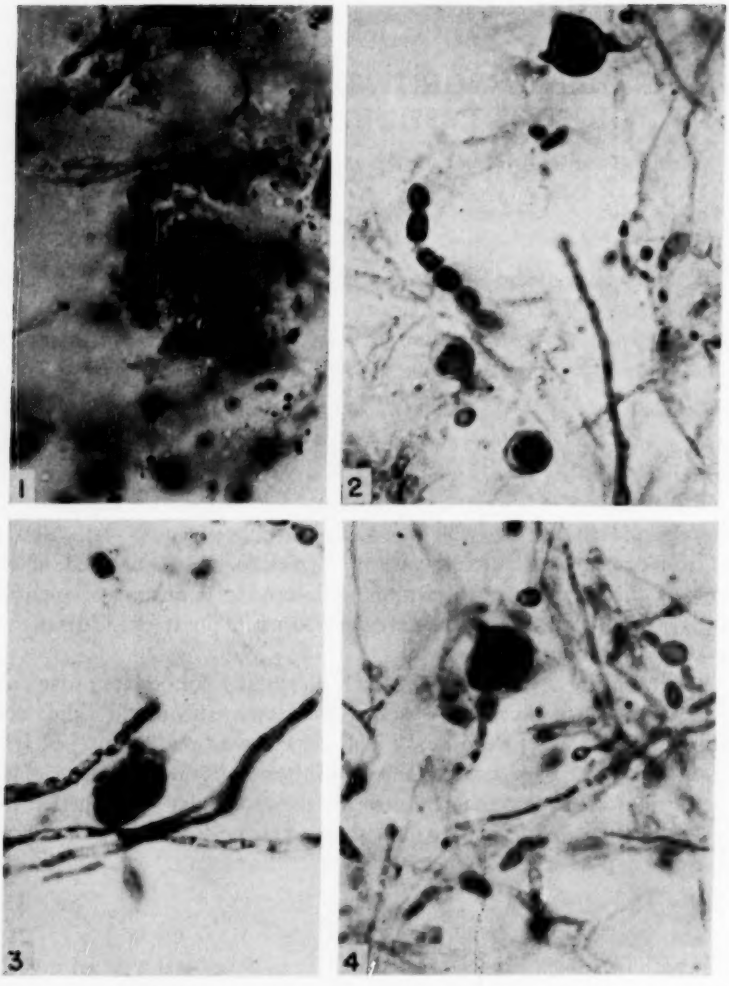


FIG. 1. *Coccidioides immitis* strain *973*. A single culture spherule showing several well differentiated endospores. Photomicrograph × 1236. FIG. 2. *Coccidioides immitis* strain *2082*. Showing culture spherules containing solid protoplasmic masses and arthrospores. Photomicrograph × 945. FIG. 3. *Coccidioides immitis* strain *973*. A single culture spherule and vacuolated mycelium. Photomicrograph × 945. FIG. 4. *Coccidioides immitis* strain *2082*. A spherule with intact cell wall surrounded by unusual hyphal formations. Photomicrograph × 945.

like structures many of which contained endospores (FIGS. 1, 3, 4). These so-called culture spherules were smaller than the true spherules seen in the exudates and tissue of man and animals or in the yolk sac of embryonated eggs. A few arthrospores could be seen in cultures of strain *2082* only after an extensive search (FIG. 2).

Attempts to induce consistently the formation of culture spherules in strains of *C. immitis* by means of "Tween 80" have failed. All strains, however, show a marked reduction in arthrospore formation and some showed many large, dark-staining bodies along the course of the mycelium.

DISCUSSION

The possible effect of detergents or a wetting agent such as "Tween 80" on the growth of fungi is not known. In the experiments described in this report, strains of *C. immitis* showed a loss of or a great reduction in arthrospore formation after a brief exposure in a saline "Tween 80" mixture. Two strains also developed the so-called culture spherules.

A medium containing oleic acid has been reported by Negroni (**6**) to prevent sporulation in many strains of *C. immitis*. Since "Tween 80" is an ethylene ester of oleic acid, the non-sporulating behavior of the strains discussed in this report might seem to corroborate Negroni's observation. However, a check of one of his cultures, strain *694* in which he described peculiar enlarged hyphal bodies (esclerotia rudimentarios), has revealed it to be a species of *Scopulariopsis*.

The use of "Tween 80" or similar materials to induce non-sporulating cultures of *C. immitis* may find practical application in certain lines of research. When large quantities of arthrospores are needed, however, it might prove necessary to avoid the use of such materials. Although cultures showing Tween-induced pleomorphism can be reverted by subculturing, it takes several generations to reclaim an adequately sporulating culture.

SUMMARY

Several strains of *C. immitis*, when exposed to saline "Tween 80" solutions, were found to exhibit pleomorphism in the following manner: moist mycelial growth on the agar surface, varying degrees of reduced arthrospore formation and the appearance of culture spherules in some strains.

DEPARTMENT OF BACTERIOLOGY,
SCHOOL OF MEDICINE,
DUKE UNIVERSITY

REFERENCES

1. **Baker, E. E. and E. M. Mrak.** Spherule formation in culture by *Coccidioides immitis* Rixford & Gilchrist. Am. Jour. Trop. Med. **21**: 589–594. 1941.
2. ——, **E. M. Mrak and C. E. Smith.** The morphology, taxonomy and distribution of *Coccidioides immitis* Rixford & Gilchrist 1896. Farlowia **1**: 199–244. 1943.
3. **Burke, R. C.** In vitro cultivation of the parasitic phase of *Coccidioides immitis*. Proc. Soc. Exper. Biol. & Med. **76**: 332–335. 1951.
4. **Lack, A. R.** Spherule formation and endosporulation of the fungus *Coccidioides* in vitro. Proc. Soc. Exper. Biol. & Med. **38**: 907–909. 1938.
5. **Looney, J. M. and T. Stein.** Coccidioidomycosis. The hazard involved in diagnostic procedures, with report of a case. New England Jour. Med. **242**: 77–82. 1950.
6. **Negroni, P.** Estudios sobre el *Coccidioides immitis* Rixford & Gilchrist. III. Estudio micologico comparativo de las cepas argentinas y norteamericanos. Revista del Instituto Bacteriologico "Malbran" **14**: 136–147. 1949.
7. **Smith, D. T. and E. R. Harrell, Jr.** Fatal coccidioidomycosis. A case of a laboratory infection. Am. Jour. Tuber. **57**: 368–374. 1948.
8. **Vogel, R. A. and N. F. Conant.** Cultivation of *Coccidioides immitis* in the embryonated hen's egg. Jour. Bact. **64**: 83–86. 1952.

STUDIES OF THE CREOSOTE FUNGUS, HORMODENDRUM RESINAE[1, 2, 3]

DAVID H. MARSDEN

(WITH 7 FIGURES)

In 1942 there appeared in the literature an interesting account of a fungus whose common habitat is wood that has been impregnated with creosote or coal tar (**3**). Although the fungus did not appear to impair the strength of the wood, such an extraordinary resistance to highly toxic compounds is in itself sufficient incentive to further inquiry into the biology of the fungus possessing it. Moreover, when that fungus is found commonly in poles and railroad ties, which are used in such great numbers as to constitute a considerable drain on our forest resources, it becomes apparent that the situation may be of economic importance too. A question arises whether or not the fungus may in some way have a deleterious effect on the preservative intended to protect the wood against decay. This paper reports on some aspects of the biology of this singular mold.

MORPHOLOGY AND GROWTH CHARACTERISTICS

On nutrient agar media: The morphology and development of *H. resinae* on artificial culture media have been adequately described by Christensen *et al.* (**3**). In the present studies it was found that the mold will grow and sporulate well on malt extract agar, potato dextrose agar, a modified Sabouraud's agar, and a basal mineral-glucose agar with yeast extract. Undoubtedly reasonably good growth could be obtained on other natural and synthetic media as well. For purposes of com-

[1] A portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Harvard University. This study was initiated under tenure of an American Creosoting Company Fellowship at Harvard.

[2] The author is deeply grateful to Doctor W. H. Weston for his guidance and counsel during performance of the research and in preparation of the manuscript and to Doctor M. A. McKenzie, who not only assisted in editing the manuscript but also encouraged an early completion of the author's project following his residence at Harvard.

[3] Contribution No. 873 of the Massachusetts Agricultural Experiment Station.

parison the growth on malt extract agar is considered here to be the "normal" type.

In creosoted wood: The fungus was observed in microsections of wood from creosoted, southern pine telephone poles. In this habitat the hyphae are conspicuous, septate, smooth or verruculose, and vary in color from light brown to dark brown. They tend to be massed in the wood rays, but frequently pass longitudinally in the tracheid lumina. The hyphae have not been observed to penetrate tracheid walls by "boring," as do

FIG. 1. Conidiophore of *H. resinae* inside the lumen of a tracheid of southern pine wood, × 1164.

the wood-rotting Basidiomycetes, but pass from cell to cell via the pit orifices, as is common with other molds inhabiting wood.

Conidiophores and conidia are produced on the surface of the wood and within the wood elements (FIG. 1). The fruiting structures produced within tracheid cavities are of necessity reduced in size and prolificness, but otherwise they are similar to those produced on the surface of wood and in nutrient agar cultures. This production of elaborate conidiophores within the confines of wood cells is certainly unusual and

is probably dependent on a precise balance of moisture and aeration. Cursory attempts to reproduce this phenomenon in small blocks of treated wood inoculated in the laboratory were not successful.

METHODS AND MATERIALS

Isolation of the fungus: For the present investigations, *H. resinae* was isolated in pure culture from creosoted poles by placing chips of wood from the poles on malt extract agar medium containing 1.0 percent creosote. Usually within a week the mold had grown and sporulated abun-

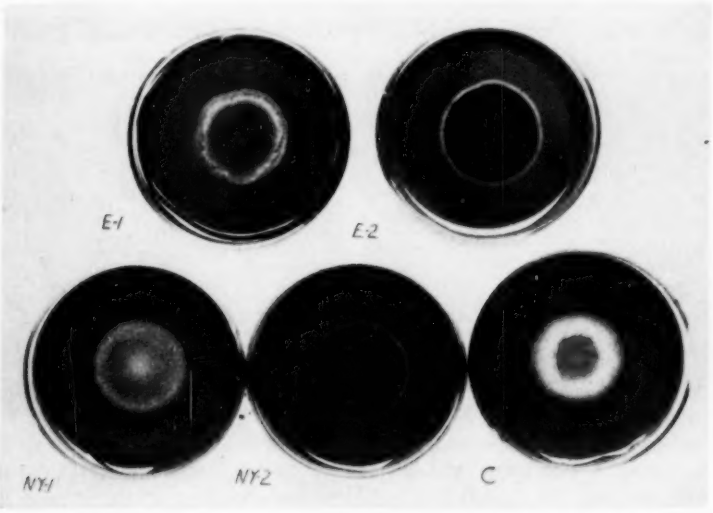


FIG. 2. Strains of *H. resinae* on malt-creosote agar. E-1 = Enola isolate, E-2 = Enola saltant; NY-1 = New York isolate, NY-2 = New York saltant; C = Christensen isolate.

dantly on the surface of the chips and on the medium. No special precautions needed to be taken against contamination with creosote in the medium. By this method isolates of *H. resinae* were obtained from several treated and partially decayed poles from Georgia, Massachusetts, New York, Pennsylvania, and Santo Domingo.

Strains of the fungus: Among the several isolations of *H. resinae* from creosoted wood, two distinct morphologic strains were obtained. Both of these strains differed from a culture of the mold sent to the author by Dr. C. M. Christensen. Moreover, the two strains obtained directly

from creosoted timber saltated in culture, thereby producing two additional strains which have been carried unchanged on several types of media, and through several transfers, for more than two years. For identification purposes the strains will hereafter be designated according to their origin. For instance, the strain isolated from a pole from New York will be called the "New York isolate," and the form it produced by saltation will be called the "New York saltant." The Enola isolate was obtained from a piece of pole from Enola, Pennsylvania. The Christensen isolate was generously supplied by Dr. C. M. Christensen of the University of Minnesota.

Morphological differences among these strains are most easily distinguished in cultures on malt extract agar in Petri dishes. Gross varia-

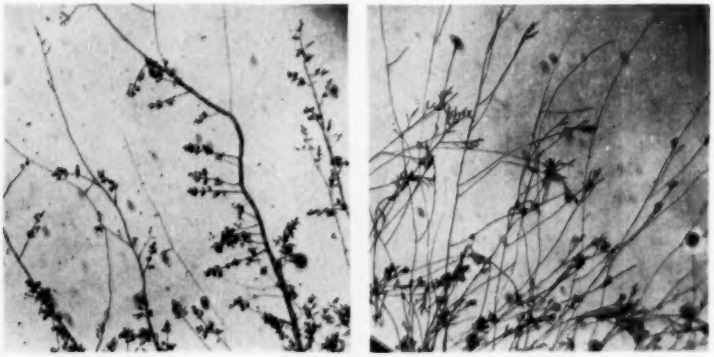


FIG. 3. Strains of *H. resinae*. L. New York isolate strain. R. New York saltant strain. Note longer and less prolific conidiophores of the saltant strain. 72-hour slide cultures, stained with methylene blue, × 100.

tions are also apparent on malt extract-creosote agar (FIG. 2). The distinguishing characters include the color of the spores en masse, the reverse color of Petri dish cultures and the relative length and prolificness of the conidiophores. The New York isolate has brown spores and the color of the culture in reverse is brown. The Enola isolate has olive-green spores with the reverse color of the culture dark blue. The Christensen isolate has brown spores with the reverse color blue. The two saltant strains differ from their parent strains in having longer and less prolific conidiophores (FIG. 3).

Sources of special materials: The creosote samples used in some tests described here were all Barrett brand, A.W.P.A. (American Wood-Preservers Association) No. 1 distillate creosote oil obtained in gallon lots from five Barrett plants. The samples are identified by their place

of origin, i.e., Chicago, Philadelphia, Syracuse, Malden, and Edgewater.

The fractional distillation samples of creosote were supplied by Dr. W. H. Fulweiler, Chemical Engineer, Philadelphia.

The tar acids, tar bases, and hydrocarbons, all of technical or practical grade, were obtained locally.

The test methods: Since certain procedures were common to each experiment they will be described now. Unless otherwise specified, the following methods were used: (1) 2.0 percent agar was used to solidify the media, (2) media were sterilized by autoclaving 20 minutes at 15 pounds pressure, (3) creosote, coal tar, water gas tar, acids, bases and hydrocarbons were not autoclaved but were added to the warm, sterile agar, (4) liquid constituents were measured by volume, not by weight, (5) 25.0 ml of medium were measured into each of three Petri dishes for each test, (6) each test culture of *H. resinae* was initiated by picking up spores of the fungus on a 3 mm disk of water agar or creosote agar and planting the disk in the center of the culture dish, (7) cultures were incubated at 29° C, (8) colony diameters were averaged after measuring each with a draftsman's compass and a millimeter scale.

CARBON SOURCES

The ability of *H. resinae* to utilize various compounds as sole sources of carbon was determined by incorporating these compounds singly into a basal mineral medium containing 1.4 g K_2HPO_4, 0.6 g Na_2HPO_4, 0.25 g $MgSO_4 \cdot 7H_2O$, 4.0 g NH_4NO_3, 20 g agar, 1000 ml distilled water. The carbon sources were chosen to include a monosaccharide, a disaccharide, a trisaccharide, a polysaccharide, an alcohol, and a few complex carbon-containing compounds. Three percent by weight or volume was added to the basal medium. The New York isolate of *H. resinae* was used as inoculum.

(1) Glucose—Growth and sporulation dense and rapid.
(2) Sucrose—Limited mycelial development; hyphae diffuse, lightly pigmented, subsurface; conidiophores and conidia few and mostly subsurface. Sucrose apparently not readily utilized.
(3) Raffinose—Similar to growth on the sucrose medium.
(4) Cellulose (pulped filter paper)—No significant growth.
(5) D-mannitol—Growth restricted as on sucrose medium.
(6) Lignin (from spruce and aspen wood derived by the cold, 72 percent sulfuric acid method)—Dense, rapid growth and sporulation comparable to that with malt extract or glucose as the carbon source.

(7) Coal (pulverized bituminous)—Diffuse, restricted growth.

(8) Petroleum (automotive lubricating oil)—No significant growth.

NITROGEN SOURCES

The ability of *H. resinae* to utilize various compounds as sole sources of nitrogen was determined by incorporating these compounds singly into a glucose-mineral-agar medium. The basal medium was the same as that described in the section on *Carbon Sources,* with the exception that 3 percent glucose was added and the nitrogen source was varied. The nitrogen compounds were chosen to include a nitrite, a nitrate, an ammonium radical, ammonium and nitrate combined, and organic compounds. The nitrogen level was adjusted to 0.14 percent N, whenever possible, this being the equivalent of the 0.4 percent NH_4NO_3 used as the nitrogen source in some other experiments described here. The nitrogen content of the peptone was unknown; the same weights of peptone and urea were used. The New York isolate of *H. resinae* was the inoculum.

TABLE I

UTILIZATION OF VARIOUS NITROGEN SOURCES BY H. RESINAE

N source	Percent of N level	Av. colony diam. in mm 3 Petri dishes—7 days	Remarks
$NaNO_2$	0.14	9	Very poor growth
$NaNO_3$	0.14	28	Diffuse hyphae; sparse sporulation
$(NH_4)_2SO_4$	0.14	30	Good growth and sporulation
NH_4NO_3	0.14	38	Good growth and sporulation
Urea	0.14	0	No growth
Urea (acidified)	0.14	15	Diffuse hyphae; sparse sporulation
Peptone	(N level unknown) (3 g/l of peptone)	48	Excellent growth and sporulation

TABLE I lists the average colony diameters attained by the mold with the various nitrogen sources. The data indicate that nitrite-N, nitrate-N, and urea-N are not readily metabolized as sole nitrogen sources, whereas ammonium-N and peptone-N support normal growth and sporulation. Although the fungus spread quite rapidly on the nitrate medium, and to a lesser extent on the acidified urea medium, the mycelia were diffuse and largely subsurface, with relatively few spores; the cultures appeared to be nitrogen-starved.

EFFECT OF CONCENTRATION OF CARBON OR NITROGEN IN THE MEDIUM

The use of solid media and spore-mass inoculations for these nutrition studies posed a problem of accurate interpretation of results. Under these conditions, some of the spores may germinate and grow even on a nutritionally unfavorable medium, probably at the expense of the nutrients contained in the spore mass. Moreover, in this circumstance of virtual starvation the fungus makes a rapid but diffuse growth through the medium, apparently in search of the vital sustenance which has been withheld. Therefore, the rapidity and extent of mycelial development was not a reliable criterion of the nutritional capacity of the fungus. In this instance this difficulty could not be avoided by comparing dry weights of mycelia from liquid cultures because certain insoluble and immiscible

TABLE II

EFFECT OF CONCENTRATION OF C OR N ON H. RESINAE ON AGAR MEDIA

Ppm C	Ppm N	Av. colony diam. in mm 3 Petri dishes—7 days	Sporulation
0	1400 (0.4% NH_4NO)	28	Density of sporulation increasing slightly
10	1400	35	
40	1400	43	
80	1400	48	
120	1400	53	
240	1400	60	
12,000 (3% glucose)	0	37	Density of sporulation increasing markedly
12,000	9	37	
12,000	18	37	
12,000	36	37	
12,000	72	37	

substances (creosote, coal tar, water gas tar) were to be included as sole carbon or nitrogen sources. Although it is possible to emulsify these preservatives with water by adding gum arabic, it is not possible to maintain these emulsions without constant, violent agitation. However, it is easy to prepare permanent homogeneous emulsions of these oily materials by solidifying the emulsions quickly with agar. This technique, which will be described more fully later, was the one used.

The question of interpreting results under these circumstances was resolved by taking both colony diameter and density of sporulation together as a measure of nutritional capacity. That is, the more nearly a culture approached, in density of growth and sporulation, the appearance of *H. resinae* on 3 percent malt extract agar the more readily the par-

ticular nutrients in the medium were utilized. Results obtained in the foregoing experiments on carbon and nitrogen nutrition appeared to justify this interpretation. However, to prove the validity of this procedure an additional experiment was performed as follows.

Two series of cultures were prepared: one with increasing amounts of glucose in a basal mineral medium, the other with increasing amounts of NH_4NO_3 in a basal mineral-glucose medium. The New York isolate of *H. resinae* was used as inoculum. TABLE II indicates the results of these tests.

Two points were demonstrated clearly in this experiment: (1) within the limits of the concentrations used, the amount of available carbon in the medium has a direct correlation with the rate of radial growth of the fungus, and, when N is not a limiting factor, with the density of sporulation; (2) within the limits of the concentrations used, the amount of available nitrogen in the medium has no effect on the rate of radial

TABLE III

CREOSOTE AS CARBON AND NITROGEN SOURCE FOR H. RESINAE

Carbon source	Nitrogen source	Incubation time—days	Av. colony diam. in mm 3 Petri dishes	Remarks
Glucose	NH_4NO_3	11	55	Good sporulation
Glucose and creosote	NH_4NO_3 and creosote	14	20	Good sporulation
Creosote	NH_4NO_3	14	12	Fair sporulation
Glucose	Creosote	14	24	Fair sporulation

growth of the fungus but has a marked effect on the density of sporulation. It was observed in another series of cultures that as little as 175 ppm of nitrogen in the medium is enough to permit normal growth and sporulation as compared with the development of the mold on 3 percent malt extract agar. Accordingly, it may be concluded that rate of growth and density of sporulation together may be used as a measure of carbon utilization by *H. resinae*, whereas density of sporulation alone may be used as a measure of nitrogen utilization by the mold.

CREOSOTE AS CARBON OR NITROGEN SOURCE

A natural question about a fungus that not only manifests an uncommonly high tolerance for coal tar and creosote, but also will germinate and grow in the undiluted materials, is whether or not that fungus can metabolize these wood preservatives. To answer this question, *H. resinae* was inoculated onto a basal mineral agar medium with creosote

added as the sole carbon source or nitrogen source. One percent by volume of creosote was blended into the warm, sterile agar medium with a Waring Blendor. Higher concentrations of creosote were not used because in the absence of a complete medium for growth, the mold is too greatly inhibited by the creosote. The New York isolate was the inoculum.

The results of this test are indicated in TABLE III.

The test showed that 1 percent creosote as the sole carbon source supports a restricted growth with fair sporulation; as the sole nitrogen source, it supports a more extensive mycelial development with fair sporulation. The density of sporulation in either case is not equal to that on a 1 percent creosote medium with supplementary sources of C and N added, but is greater than that on media to which no C or N compounds are added.

TABLE IV

COAL TAR AND WATER GAS TAR AS C OR N SOURCES FOR H. RESINAE

Carbon source	Nitrogen source	Av. colony diam. in mm 3 Petri dishes —14 days	Remarks
Glucose and coal tar	NH_4NO_3 and coal tar	47	Good sporulation
Coal tar	NH_4NO_3	19	Fair sporulation
Glucose	Coal tar	36	Fair sporulation
Glucose and water gas tar	NH_4NO_3 and water gas tar	61	Good sporulation
Water gas tar	NH_4NO_3	19	Poor sporulation
Glucose	Water gas tar	25	Poor sporulation

Probably all the constituent compounds of creosote contain the C atom and are therefore potential sources of carbon. However, the restricted growth with creosote as the sole C source would seem to indicate that most of this carbon is not readily metabolized by the fungus. The potential N sources in creosote are more limited in number and include such compounds as pyridines (C_5H_5N), quinolines (C_9H_7N), and acridines ($C_{13}H_9N$). At least some of these nitrogen compounds would appear to be metabolized by *H. resinae*.

COAL TAR AND WATER GAS TAR AS THE CARBON OR NITROGEN SOURCE

The growth of *H. resinae* on a basal mineral medium containing coal tar or water gas tar as the sole C or N source was determined by blending 4 percent by volume of either of the preservatives into the warm, sterile agar medium. Because coal tar and water gas tar are not nearly

so toxic as creosote, it was possible to include a relatively high percentage (4 percent) of these compounds without inhibiting growth of the fungus too greatly. This was an advantage in that more of the metabolizable constituents would be available to the fungus. TABLE IV indicates the results of these tests.

These data are interpreted to mean that coal tar may serve as a carbon or nitrogen source for *H. resinae*. Although the radial growth of the fungus is restricted, the density of sporulation approaches that on a coal tar medium containing supplementary sources of carbon and nitrogen. On the other hand, water gas tar supports only feeble growth and sporulation of the fungus, despite the fact that water gas tar is less toxic than coal tar and far less toxic than creosote.

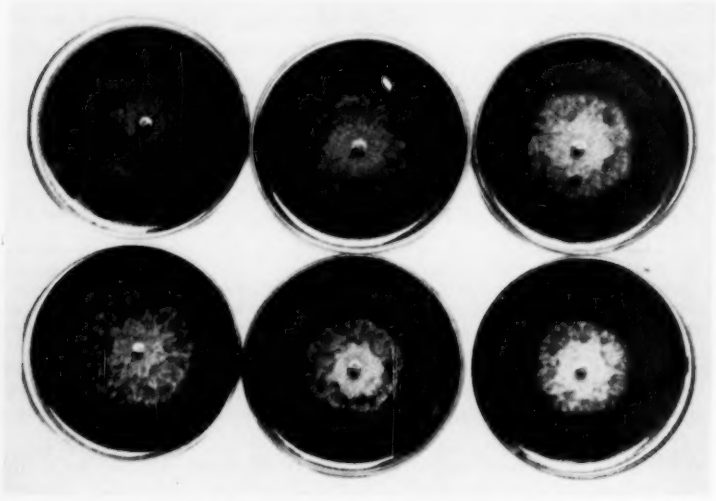


FIG. 4. Coal tar as the nitrogen source for *H. resinae:* Effect of increasing concentrations of coal tar. Note the greater density of sporulation as the amount of coal tar in the medium is increased in increments of 1.0 percent, from 1.0 percent (top left) to 6.0 percent (bottom right). 14-day cultures.

INCREASING CONCENTRATION OF COAL TAR AS THE NITROGEN SOURCE

According to the preceding experiment, coal tar may be utilized by the mold as a sole source of nitrogen. Therefore, it might be expected that varying the concentration of coal tar in the medium would result in differences in the growth of the fungus. To test this thesis the fungus

was grown on a basal mineral-glucose medium in which different amounts of coal tar were included as the nitrogen source. The results of this test are shown in FIG. 4. Increasing amounts of coal tar in the medium did have a favorable effect on the density of mycelial growth and sporulation. It seems reasonable to assume that the enhanced growth and sporulation were due to the additional N available to the fungus.

RESISTANCE TO TOXICITY

The following series of experiments were designed to learn more about the toxicity of creosote to the fungus and the mold's resistance to the toxic principals of creosote.

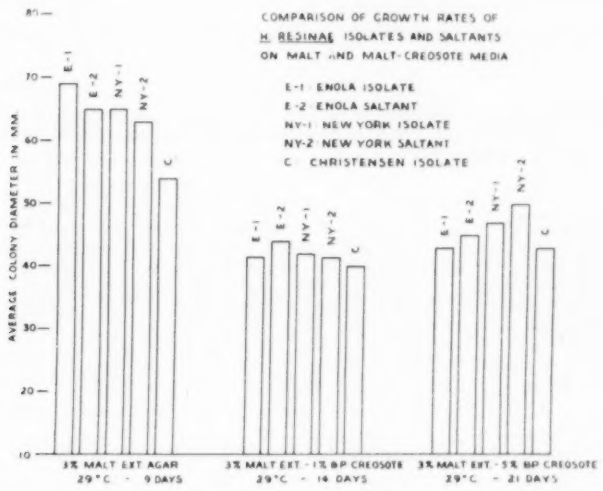


FIG. 5. Relative growth rates of *H. resinae* strains on malt and malt-creosote media.

Strain tolerance of creosote: To choose the strain of *H. resinae* most tolerant of creosote for certain experiments that were to follow, the five strains of the fungus were compared on creosote-nutrient agar. Barrett (Philadelphia) creosote was emulsified with water by using 1.0 g of gum arabic per ml of creosote and working up the emulsion with mortar and pestle. Appropriate amounts of this emulsion were added to sterile 3.0 percent malt extract agar. FIG. 5 is a graphic representation of the growth responses of the several strains with 1.0 percent creosote and with 5.0 percent creosote in the medium. The average colony diameters attained by the fungus on 3.0 percent malt extract agar are included also.

It may be seen that although the Enola isolate was the fastest growing strain on plain nutrient agar, particularly exceeding the growth rate of the Christensen isolate on this medium, nevertheless the Enola isolate was equalled or exceeded in growth by all other strains on the nutrient medium containing 5.0 percent creosote. The New York strains appeared to be slightly more tolerant of a high concentration of creosote than were the others.

The superior tolerance of the New York strains to creosote was confirmed in another test involving creosotes of different origins. Coal tar creosote is known to differ in chemical composition from one batch to another, and more especially among samples from different sources of supply.

In this test the several strains of *H. resinae* were compared on five samples of creosote of different origins. The medium consisted of 3.0

TABLE V

AVERAGE COLONY DIAMETERS OF H. RESINAE STRAINS ON NUTRIENT AGAR CONTAINING 1.0 PERCENT CREOSOTE OF DIFFERENT ORIGINS

Fungus strain	Brand of Barrett creosote Av. colony diam. in mm in 14 days					
	Chicago	Philadelphia	Syracuse	Edgewater	Malden	Average
New York isolate	41	40	37	45	43	41.2
New York saltant	40	43	37	42	43	41.0
Enola isolate	34	39	34	39	31	35.4
Enola saltant	38	39	38	42	36	38.6
Christensen isolate	34	41	39	45	43	40.4
Average	37.4	40.4	37.0	42.6	39.2	

percent malt extract agar with 1.0 percent emulsified creosote included. TABLE V indicates the average colony diameters attained by the fungus strains on these media.

Creosote-nutrient agar: The effect of creosote on the growth and appearance of *H. resinae* was determined by growing the fungus on malt extract agar containing various concentrations of the preservative. Barrett (Philadelphia) creosote was blended into hot, 3.0 percent malt extract agar with a Waring Blendor. Both the New York isolate and the Enola isolate of *H. resinae* were used.

The inhibition of the New York isolate by creosote concentrations ranging from 0.0 to 16.0 percent and of the Enola isolate by creosote concentrations of 0.0 to 8.0 percent is illustrated graphically in FIG. 6.

For both strains of the fungus the curve of inhibition is nearly a straight line up to 0.5 percent creosote in the medium. The steepness

of the curve declines appreciably between 0.5 and 2.0 percent creosote, and then nearly levels off for the higher concentrations. There is little difference in inhibition of growth between 2.0 and 8.0 percent with the Enola strain or between 2.0 and 16.0 percent with the New York strain. The decline in unit toxicity of the creosote with increasing amounts added may perhaps be attributed to the low solubility of the preservative in water. The greater, though unproportional, inhibition of the fungus by the higher concentrations might be the result of physical alterations in the medium produced by the relatively large amounts of creosote oil

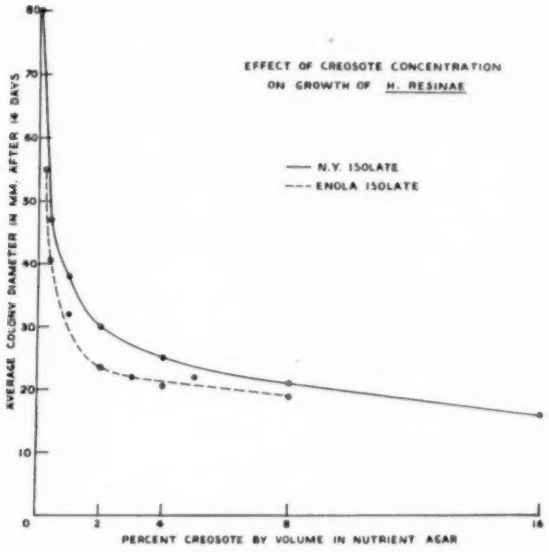


FIG. 6. Effect of creosote concentration on strains of *H. resinae*.

included. That this may be so is attested by the moist colony formation and delayed sporulation that occurs when more than 2.0 percent creosote is included in the medium.

Comparison of the toxicity of creosote to H. resinae and a wood-rotting fungus: The relative toxicity of creosote to *H. resinae* and a wood-rotting Basidiomycete was determined by comparing the growth rates of two strains of the mold, on malt extract media containing several different creosotes, with the growth rates of the Basidiomycete, Madison *517*, on media containing the same creosotes. The fungus, Madison *517*, obtained from the Forest Products Laboratory, Madison, Wisconsin, is a

Basidiomycete used commonly in toxicity assays of wood preservatives (**10**). The concentration of each creosote that inhibited growth of Madison *517* was determined by inoculating series of test tube slants of creosote-nutrient agar, each series comprising a range of concentrations of the particular creosote sample used.

FIG. 7 illustrates graphically the effect of the several creosotes on the mold and on the wood-rotting fungus. The values for the two strains of the mold, taken from the data in TABLE V, are colony diameters attained in 14 days on 3.0 percent malt extract–1.0 percent creosote agar media. On the other hand, the growth rates of Madison *517* were determined on 3.0 percent malt extract–0.1 percent creosote agar media since

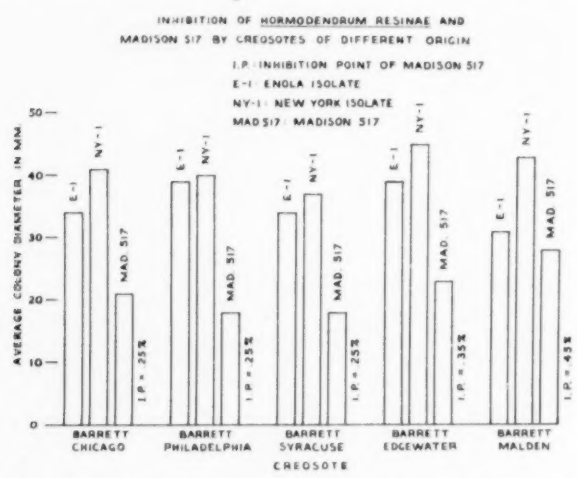


FIG. 7. Relative effects of different creosotes on *H. resinae* and a wood-rotting fungus.

much higher concentrations of creosote would inhibit this fungus too greatly.

It is apparent that there is little correlation between the growth rates of the mold and of Madison *517* on these media or between the growth rates of the mold and totally inhibitory concentrations of the creosote to Madison *517*.

The lack of correlation between the relative growth rates of the New York strain and the Enola strain of *H. resinae* on these media is further evidence of physiological differences between the two strains.

Toxicity of distillation fractions of creosote: Coal tar creosote is not a single chemical substance but rather a complex mixture of several

hundred compounds. The known constituents comprise three principal classes, namely, tar acids, tar bases, and hydrocarbons.

The fractional distillation of a mixture of compounds having different boiling points is almost certain to yield fractions with different degrees of toxicity to fungi. It has generally been found that the mixture that distills between 200° and 275° C is more toxic to wood-destroying fungi than whole creosote (**5**). Moreover, Bateman (**1**) has shown that the toxicity of a given creosote is closely related to the percentage of the 200° to 275° C fraction included.

The effect of fractional portions of creosote on *H. resinae* was determined by incorporating separately several distillate fractions into malt extract agar medium. The distillates were heated to dissolve sediments, and 1.0 percent by volume was blended into warm, sterile, malt extract agar with a Waring Blendor. Both the New York isolate and the New York saltant strains of *H. resinae* were tested against the distillates. TABLE VI indicates the results of these tests.

TABLE VI

INHIBITION OF H. RESINAE BY DISTILLATION FRACTIONS OF CREOSOTE

Distillation fraction	Av. colony diam. in mm of *H. resinae* in 14 days on 1 percent creosote—3 percent malt ext. agar	
	N. Y. isolate	N. Y. saltant
Control	90	90
Whole creosote[1]	41	41
210°–235°C	14	14
235°–270°C	14	14
270°–315°C	26	24
315°–355°C	47	46
Residue	80	80

[1] Average for five creosotes of different origin.

The data indicate that the fractions distilling between 210° and 270° C are more inhibitory to both strains of *H. resinae* than whole creosote or any of the other fractions. This observation is in accordance with what has been found for wood-rotting fungi and indicates that, despite the unusual tolerance of *H. resinae* for creosote, this mold nevertheless responds to the toxicity of the several distillation fractions of creosote to the same relative degree as do the wood-rotters.

Toxicity of some tar acids, tar bases, and hydrocarbons of creosote: The toxicity of creosote is inherent in certain of its numerous component compounds. Of these compounds some are low in potential toxicity or else are insufficiently soluble in water to exert their full poisonous effects. It has been mentioned previously that coal tar creosote is comprised

chiefly of three classes of compounds: tar acids, tar bases, and hydrocarbons. The so-called tar acids include various phenols, cresols, and naphthols, all of which are highly toxic to wood-destroying fungi. The tar bases include pyridines, quinolines, and acridines, most of which are also toxic to fungi. The hydrocarbons, which constitute most of the volume of creosote, include benzene, toluene, anthracene, xylene, acenaphthene, fluorene, naphthalene, and phenanthrene among others. Of these, some are known to be very effective toxicants, whereas others release their poisons slowly or add desirable physical properties to the over-all preservative value of creosote.

TABLE VII

GROWTH OF H. RESINAE ON NUTRIENT MEDIA CONTAINING INDIVIDUAL TAR ACIDS, TAR BASES, OR HYDROCARBONS OF CREOSOTE

Compound	Boiling point °C	Percent conc.	Av. colony diam. in mm 7 days—3 Petri dishes		
			H. resinae N. Y. isolate	*Aspergillus niger*	*Cladosporium herbarum*
Tar acids					
Phenol	182	0.25	0	0	0
a naphthol	288	0.25	0	0	0
B naphthol	295	0.25	0	0	0
Tar bases					
Pyridine	115	0.25	42	52	4
Quinoline	237	0.25	0	0	0
Hydrocarbons					
Phenanthrene	340	1.0	45	9	0
Acenaphthene	277	1.0	43	21	0
Anthracene	354	1.0	45	25	9
Naphthalene	218	1.0	29	0	0
Fluorene	295	1.0	41	19	0
Control					
Malt ext. agar	—	3.0	52	37	10

The relative toxicity of some of the known constituents of creosote to *H. resinae* was determined by growing the mold on malt extract agar containing certain of the compounds of each class. The tar acids and the tar base, pyridine, were sufficiently soluble to be dissolved directly into the warm, sterile nutrient agar. The tar base, quinoline, and the hydrocarbons, all solids, were blended into the warm, sterile, nutrient agar with a Waring Blendor. The New York isolate of *H. resinae* was the inoculum, and for purposes of comparison with other mold fungi, identical tests were run with *Aspergillus niger* and *Cladosporium herbarum*.

Aspergillus niger was chosen because it is capable of survival in severely adverse environments, and also because very extensive physiological studies have been made of this mold. *Cladosporium herbarum* was chosen as the most ubiquitous member of the form-genus to which *H. resinae* is closely allied. The results of these tests are listed in TABLE VII.

The data in TABLE VII indicate that all the tar acids tested were highly toxic to the three molds. Of the two tar bases, quinoline completely inhibited the growth of all three species, whereas pyridine was only slightly toxic to *H. resinae*, somewhat more toxic to *C. herbarum*, and actually stimulatory to *A. niger*. This was the only instance in which *A. niger* manifested a greater tolerance for any of these compounds than did the *Hormodendrum*. Among the hydrocarbons, only naphthalene effected more than a slight retardation of growth of *H. resinae*. In comparison, *A. niger* was inhibited completely by naphthalene and slowed considerably by the other four compounds. *Cladosporium herbarum* was inhibited completely by all except anthracene. This morphologic ally of *H. resinae* apparently does not share the latter's remarkable tolerance for creosote and its constituent compounds.

It appears, therefore, that the unique physiology of *H. resinae*, as far as resistance to toxicity of creosote is concerned, lies in the tolerance of the mold for certain of the hydrocarbon constituents. For instance, naphthalene has been reported by Bateman and Baechler (**2**) to be totally inhibitive to the Basidiomycete, Madison *517*, at a concentration of 0.003 percent at 32° C and to permit only "spore growth" at 28° C. In contrast, *H. resinae* grew and sporulated quite readily at 28° C in the presence of 1.0 percent naphthalene. Similarly, Bateman and Baechler (**2**) found that a saturated solution of acenaphthene permitted only feeble growth of Madison *517*, whereas in the presence of 1.0 percent acenaphthene *H. resinae* made vigorous growth.

EFFECT OF H. RESINAE ON THE TOXICITY OF CREOSOTE

Whether or not a mold that lives in creosoted wood and has an ability to utilize creosote in its metabolism has any deleterious effect on the preservative is a question of practical importance.

An *in vitro* experiment was performed to determine the extent to which the toxicity of creosote might be changed by the mold. The method followed, which involved growing the mold in a creosote-nutrient emulsion prior to using the emulsion as a substrate for a wood-rotting Basidiomycete, is admittedly cumbersome and thereby prone to error.

However, it was the best that could be conceived, and the several steps were carried through with strict attention to precision and uniformity. The detailed procedure of the experiment was as follows: twelve flasks, each containing 25 ml of 2 percent malt extract–0.9 percent emulsified creosote, were sterilized and then inoculated with 0.5 ml of a spore suspension of the New York isolate of *H. resinae*. An equal number of flasks containing the same medium, but inoculated with sterile water, were prepared as controls. Gum arabic was used to emulsify the creosote with water. Both sets of flasks were plugged with cotton and capped with aluminum foil to retard evaporation. Both sets of flasks were incubated at 29° C and were agitated twice a day for the first two weeks and twice a week thereafter. At the beginning of the test period, and at five 20-day intervals thereafter, the contents of two control flasks and two inoculated flasks were removed for toximetric analyses of the creosote media. The contents of each test flask were whirled briefly in a Waring

TABLE VIII

EFFECT OF H. RESINAE ON THE TOXICITY OF CREOSOTE

No. days *H. resinae* grown in creosote	Av. colony diam. in mm of Madison *517* in 21 days on nutrient agar containing the altered creosote. Three Petri dishes	
	Test	Control
0	0	0
20	*3	*3
40	14	10
60	19	14
80	24	17
100	28	20

* Growth on inoculum disk only.

Blendor to break up the fungus mat that had formed, after which the volume of the altered medium with the fragmented fungus was brought up to the original 25 ml by adding distilled water. (Each flask lost approximately 2 ml of solution through evaporation during each 20 days of incubation.) The altered medium with the original water content thus restored was then added to 50 ml of 5 percent malt extract–3 percent agar and autoclaved 15 minutes at 15 lbs pressure. The same treatment was accorded each control flask. Thus the final product in each flask, calculating from the original 25 ml of 2 percent malt extract–0.9 percent creosote, was a 4 percent malt extract–0.3 percent creosote–2 percent agar medium. The pH of the media at this point did not in any case vary more than 0.1 point from pH 5; therefore, no adjustment of the acidity was necessary. Next, the contents of each flask were divided equally among three Petri dishes, and each dish was inoculated at its

center with a 3 mm disk of mycelium cut from a 7-day-old culture of the wood-rotting fungus, Madison *517*. These transplants of Madison *517* were incubated for 21 days at 29° C. Because this fungus, as shown in earlier experiments, is sensitive to creosote concentration, the differences in amount of growth attained in 21 days were interpreted as reflecting changes in the toxicity of the creosote caused by the action of *H. resinae*. The results of this experiment are indicated in TABLE VIII.

DISCUSSION AND CONCLUSIONS

Regarding the carbon nutrition of *H. resinae*, it is worthy of note that among the carbohydrates tested only glucose and chemically-derived lignin were utilized readily as sole carbon sources. It is well known that certain Basidiomycetes, which cause "white rots" in wood, may degrade lignin rapidly. Apparently, however, no work has been done to show that molds may metabolize lignin. Undoubtedly there is a gap between the ability to attack the lignin-cellulose complex in wood and the ability to utilize lignin freed from its complex with cellulose. Christensen *et al.* (**3**) found that no significant changes in the physical properties of wood were induced by incubation with *H. resinae*, so the mold seemingly does not utilize lignin in its native state.

On the basis of the nitrogen metabolism experiment, *H. resinae* would fall into Robbins' (**11**) category II which includes those fungi that can utilize ammonia nitrogen and organic nitrogen only. More exhaustive study of the mold's nitrogen metabolism may require a revision of this assignment.

Referring to TABLE I, it will be noted that unmodified urea medium supported no growth in 7 days, although the spores germinated and produced a limited growth in cultures held for two weeks or longer. This inability to utilize urea was unexpected inasmuch as Foster (**4**), in his comprehensive review of nitrogen nutrition among the fungi, points out that urease activity nas been found widely among both filamentous molds and the higher forms. Moreover, it has been found by several workers that with glucose as the carbohydrate source (as it was in the experiment reported here) urease activity is stimulated, leading to the rapid conversion of urea to ammonia and CO_2. This carbohydrate effect on urease activity may be an indirect one, the formation of the enzyme, urease, being induced by the reduced pH of the medium caused by organic acid formation from the sugar. It has been found that above pH 6 urease activity is always low, whereas below pH 6 urease activity is high, and urea disappears from the medium (**4**). The glucose-mineral

salts medium used in the tests reported here had an initial pH of approximately 8, a pH high enough possibly to inhibit the formation of urease by the fungus. For this reason a parallel test was made with the urea medium reduced to pH 4 by the addition of lactic acid. Although a limited amount of growth occurred on this medium in 7 days (TABLE I), the mycelial development and sporulation were poor indeed.

Also, the inability of the fungus to grow well with nitrite N deserves comment. According to Foster (**4**) and Lilly and Barnett (**7**), several workers have found that fungi utilize nitrites as sole nitrogen sources except when the medium is highly acid. Moreover, nitrite is postulated as an intermediary in the metabolism of nitrate by fungi. In these tests the nitrite medium was approximately neutral; consequently the failure to utilize the nitrite cannot be attributed to excess acidity.

The data presented in these studies indicate that *H. resinae* may utilize creosote or coal tar as a source of carbon or nitrogen to produce a fairly vigorous growth and sporulation of the fungus. This does not mean that the fungus will metabolize these noxious compounds preferentially, but it does indicate how the fungus can thrive even on freshly creosoted wood. It seems to be a situation where the toxicity of the preservative opposes the resistance and nutritional capacity of the fungus. It would be of interest to know to what extent the constituent compounds of the preservatives contribute to each side of this contest between suppression and sustenance. However, the chemistry of creosote and coal tar is so complex that it is highly improbable that the full story will ever be known.

Undoubtedly the most unique feature of *H. resinae* is its ability to thrive in high concentrations of creosote and coal tar. It is probably safe to say that with the possible exception of certain bacteria commonly inhabiting creosoted wood (**3**, **8**) no other cryptogam can grow in more than 1 percent creosote in liquid or agar media. For most fungi and bacteria the inhibition point is something less than 0.5 percent. Because of this extraordinary tolerance for creosote one may be inclined to rate the *Hormodendrum* on a par with other molds that are known to be unusually resistant to the action of toxic materials. For example, there are the molds that will grow in saturated solutions of copper sulfate (**13**), certain species of *Penicillum* tolerant of organic mercury salts (**12**, **15**), and a number of fungi tolerant of arsenic compounds (**6**, **14**). However, the tolerance of the *Hormodendrum* for creosote is a more remarkable phenomenon than any of these others. Consider that creosote is not a single compound but rather a mixture of several hundred compounds, many of which are highly toxic to most fungi. Consider, too, that no other fungus is known to tolerate a 100 percent concentra-

tion of a generally toxic material. Moreover, *H. resinae* not only is tolerant of creosote and coal tar but the mold can actually metabolize these toxic preservatives.

In an experiment reported here *H. resinae* was inhibited by distillation fractions of creosote to the same relative degree as wood-rotting fungi in general. That is to say, the mixture that distills between 210° to 270° C was more toxic than either the higher boiling fractions, the residue, or whole creosote. Accordingly, it would be expected that the compounds in creosote having the lower boiling points would be more toxic to the mold than those compounds having the higher boiling points. To a certain extent this held true with some individual tar acids, tar bases, and hydrocarbons. In its resistance to the toxicity of some hydrocarbons of creosote *H. resinae* appears to have the advantage over *Aspergillus niger* and *Cladosporium herbarum*. What advantage *H. resinae* has over wood-rotting fungi is not so clearly indicated in these tests. Apparently, the mold is more resistant than wood-rotting Basidiomycetes to naphthalene, but this fact alone would not seem to account for the much greater tolerance of the mold to whole creosote. Further experimentation along this line would be of interest.

The data regarding the effect of *H. resinae* on the toxicity of creosote are not without significance. The rather tenuous evidence gained from this single, *in vitro* experiment to prove that the fungus may degrade creosote is strengthened by the demonstrated ability of the mold to use creosote or coal tar as a carbon or nitrogen source. Any material attacked by an organism must suffer a change of some degree in its physical and chemical constitution, and an altered composition may result in decreased toxicity. An example of this phenomenon was reported by Rabanus (**9**) who observed that certain wood-decay fungi secrete sufficient oxalic acid to convert toxic copper sulfate to innocuous copper carbonate. The present studies indicate that creosote suffers a gradual decline in toxicity because of the action of *H. resinae*. However, the lack of data concerning the effect of this mold on the preservative in wood makes impossible any accurate evaluation of the practical significance of this fungal action.

SUMMARY

1. *Hormodendrum resinae* Lindau is a fungus that may commonly be isolated from wood products treated with coal tar or coal tar creosote. Five strains of the fungus are described and certain physiological variations revealed.

2. Glucose and lignin were utilized readily as sole carbon sources by the fungus. Sucrose, raffinose, d-mannitol, cellulose, bituminous coal, and petroleum were not readily utilized as sole carbon sources.

3. Ammonium sulfate, ammonium nitrate, and peptone were readily utilized as sole nitrogen sources. Sodium nitrite, sodium nitrate and urea were not readily utilized.

4. Increasing concentrations of carbon in the medium were correlated with the radial growth and the sporulation of *H. resinae* whereas increasing concentrations of nitrogen in the medium were correlated with sporulation but not with radial growth.

5. Coal tar and creosote may serve as the sole carbon or nitrogen source.

6. The sporulation of the fungus with coal tar as the sole nitrogen source is directly proportional to the concentration of coal tar in the medium.

7. The mold is inhibited to the same relative degree as wood-rotting Basidiomycetes by certain distillation fractions of creosote.

8. The mold was tested against some constituent compounds of creosote. Only with certain hydrocarbons was a greater than usual tolerance manifested.

9. A limited *in vitro* experiment indicated that the mold may deleteriously affect the toxicity of creosote.

SHADE TREE LABORATORIES,
UNIVERSITY OF MASSACHUSETTS, AMHERST

LITERATURE CITED

1. **Bateman, E. A.** 1922. Coal-tar and water-gas-tar creosote: Their properties and methods of testing. U. S. Dept. Agr. Bull. 1036: 1–114.
2. —— **and R. H. Baechler.** 1937. Some toxicity data and their practical significance. U. S. Dept. Agr. For. Prod. Lab. R 1222, p. 1–12. (Also published in Amer. Wood Preservers Assoc. Proc. 1937.)
3. **Christensen, C. M., F. H. Kaufert, H. Schmitz and J. L. Allison.** 1942. *Hormodendrum resinae* Lindau, an inhabitant of wood impregnated with creosote and coal tar. Amer. Jour. Bot. **29**: 552–558.
4. **Foster, J. W.** 1949. Chemical activities of fungi. Academic Press, New York. 648 pp.
5. **Hunt, G. M. and G. A. Garratt.** 1938. Wood preservation. McGraw-Hill, New York. 457 pp.
6. **Kaufert, F. and H. Schmitz.** 1937. Studies in wood decay. VI. The effect of arsenic, zinc, and copper on the rate of decay of wood by certain wood-destroying fungi. Phytopath. **27**: 780–788.
7. **Lilly, V. G. and H. L. Barnett.** 1951. Physiology of the fungi. McGraw-Hill, New York, Ed. 1. 464 pp.

8. **Marsden, D. H.** 1952. Studies of decayed preservative-treated wood. Thesis. Part I. Harvard University (unpublished).
9. **Rabanus, A.** 1939. Über die Saure-Produktion von Pilzen und deren Einfluss auf die Wirkung von Holzschutznitteln. Mitt. Deutsch. Forstver. **23**: 77–89.
10. **Richards, C. A.** 1937. The doubtful identity of fungus No. *517*. Amer. Wood Preservers Assoc. Proc. **1937**: 104–106.
11. **Robbins, W. J.** 1937. The assimilation by plants of various forms of nitrogen. Amer. Jour. Bot. **24**: 243–250.
12. **Scheffer, T. C. and R. M. Lindgren.** 1932. Some minor stains of southern pine and hardwood lumber and logs. Jour. Agr. Res. **45**: 233–237.
13. **Starkey, R. L. and S. A. Waksman.** 1943. Fungi tolerant to extreme acidity and high concentrations of copper sulfate. Jour. Bact. **45**: 509–519.
14. **Thom, C. and K. B. Raper.** 1932. The arsenic fungi of Gosio. Science **76**: 548–550.
15. **Verrall, A. F.** 1949. Some molds on wood favored by certain toxicants. Jour. Agr. Res. **78**: 695–703.

CULTURE STUDIES IN THE GENERA PLEOSPORA, CLATHROSPORA, AND LEPTOSPHAERIA. II

EMORY G. SIMMONS [1]

The basis of species segregation by workers in the genera *Pleospora, Clathrospora,* and *Leptosphaeria* rests, at present, upon the relative magnitude and quality of different characters of the mature perfect stage. Chief among these characters in common use (Wehmeyer, **1948**) are those of the perithecia (size, wall thickness, formation of setae); of the asci (size, shape); and of the ascospores (shape, size, septation, color). Substratum preferences and conidial connections have been used in a few instances in the circumscription of species by Diedicke (**1903**), by Noack (**1905**), by Drechsler (**1923**), and by others.

The use of data derived on the basis of these criteria has resulted in the describing and in the naming of large numbers of species in these genera. The tendency in such nomenclatorial procedure has been to describe as a new species any collection or morphologically similar group of collections which does not satisfy a previous description completely, although a great latitude in the overlapping of character similarities of the "species" being differentiated has been allowed. The primary objections to such circumscription of species in these genera are that the investigator cannot know the genetic origin and constitution of a particular specimen, that he does not know the environmental history of the collection, and that he cannot be certain always of the maturity of the critical structures. In short, it is impossible for him to be certain of the specific identity of the organism without being informed concerning the pattern of development and the innate variability of the species.

The worker in the systematics of these genera is plagued by the lack of these data. In order to approximate a natural classification of known species, he must be able to understand the condition of existence of the organisms in nature. In this respect he must find answers to such questions concerning the natural state of affairs as the following:

(1) Are all of the similar organisms found in a collection actually derived from sources (ascospores, conidia, hyphae) which are genetically identical?

[1] Present address: Quartermaster General Laboratories, Philadelphia, Pennsylvania.

(2) Are the perithecia of a given collection the product of one, of two, or of several spores or hyphae?

(3) Are single spores of a specimen capable of reproducing the species, or does the completion of the life cycle depend upon some system of heterocaryosis or of hybridization?

(4) What are the effects of such factors as temperature, moisture, light, altitude, nutrient supply, physical nature of substratum, time of year of initiation of growth, or presence of other organisms upon the development and ultimate morphology of the critical elements? Do these factors have individual specific effects upon the growth of the organism or do they act in conjunction with or in opposition to each other?

The answers to these questions and to related questions are not known in any specific case.

In order to reconcile the system of species segregation in these genera as it exists in nature and as it is described in the literature on the basis of more or less artificial criteria, it is necessary that controlled experimental work be done involving the reconstruction or approximation of the conditions prevailing in nature. For this purpose it is essential that the experiments make use of individual organisms (i.e., the progeny of single ascospores or conidia) and that as many isolates as possible of different collections of morphologically similar organisms be examined and compared. Only in this manner can a knowledge of the actual variation-potential of a species and its strains be derived and a concrete basis for species delineation in these genera be founded.

STUDIES OF PERITHECIAL STAGES

A primary purpose of the culture work reported here was to establish conditions under which mature perithecia of the species studied would be formed. Most investigators working with this group have handled only *Pleospora herbarum*, a species which apparently is capable of fruiting on a wide range of substrata. However, little or nothing is known about methods of inducing the production of perfect fruiting structures of other similar species. Experiments were set up, therefore, which made use of physical factors which would be expected to be operative on these organisms in nature, namely, an initial growth period at "summer" temperature, further growth at "winter" temperature, ample moisture in substratum, and diffuse light.

If mature perithecia and ascospores could be produced, variability in size of the ascospores from single-spore strains of these organisms could be studied. The elucidation of this problem is of fundamental impor-

tance to the understanding of species circumscription in the group. In this connection, comparisons of ascospore size between samples derived from different single perithecia from the same culture have been made.

These two lines of investigation are treated separately here. The work on production of perithecia indicates conditions under which perithecia of some species studied can be produced. The work on the comparison of size of ascospores is presented as an introduction to a type of investigation basic to the problem of establishing species limits among these fungi.

I. PRODUCTION OF PERITHECIA

A number of different media have been used by those who have worked with pure cultures of *Pleospora*. Most of these media have been based on decoctions of plant parts.

Work on *P. herbarum* has been carried out on apple decoction (Gibelli and Griffini, **1874**); on dilute grape juice and on prune decoction (Bauke, **1877**; Kohl, **1883**); on thin decoctions of onion, of date, and of horse dung (Miyabe, **1889**); on an unidentified nutrient medium (Brefeld, **1891**); on potato, on gourd pulp, and on Mayer's medium (Cavara and Mollica, **1907**); on salep agar (Klebahn, **1918**); on malt agar and pea agar (Groves and Skolko, **1944**); and on dung agar and on straw (Hughes, **1945**). Cocchi (**1931**) states that he obtained mature perithecia of *P. herbarum* on pieces of carrot, on his agar No. 5 (mineral salts as nutrients), and on Dox's medium solidified.

Diedicke (**1902**) studied cultures of *P. trichostoma* on a prune decoction medium solidified by gelatine; Halsted (**1893**) identified the substratum for growing *P. Tropaeoli* simply as slant agar tubes.

A. *Materials and Methods.*

Since the definition of a nutrient medium necessary for the production of perithecia in *Pleospora* is not supplied by the array of substrata listed, Leonian's agar (Leonian, **1924**) was used for the most part in the presently described attempts at inducing the production of perithecia. This medium, although not completely synthetic in composition, can be standardized sufficiently well to permit its duplication in any laboratory. In a few instances, noted below, 2% malt agar and filter paper cones moistened with Leonian's medium also were employed.

Attempts at inducing perithecial formation were made with cultures of all isolates listed previously[2] which grew in culture. Transfers

[2] Lists of all collections used and of cultural results for all isolates of species studied, along with descriptive material and illustrations of the species, are presented in the first paper of these studies (Simmons, **1952**).

of fungus material (either conidia or aerial mycelium) from the original isolates were made to the solidified media in Petri dishes and in test tubes.

Individual cultures were allowed to remain at room temperature (22°–26° C) until colonies had become established. Transfer of cultures then was made to a refrigerator room which remained at 5° C and which received diffuse light during daylight hours. (Data relative to the influence of light or of the absence of light on perithecial production were not obtained.) Cultures were examined frequently for indications of the formation of perithecial initials and, later, for the presence of mature asci in perithecia which appeared.

B. *Experimental Results.*

Six of the 14 isolates studied produced mature perithecia within a year under experimental conditions. These species and the conditions under which each produced perithecia are listed. A description of the perithecial stage produced in each culture is given for each species.

PLEOSPORA AMBIGUA (Berl. & Bres.) Wehmeyer (Collection No. 3)

Two subcultures of the gross isolate of this species produced mature perithecia on Leonian's agar in Petri dishes. The initial period at room temperature was 20 days. Only a few perithecia containing mature ascospores were produced within 36 weeks. The greatest number of ascospores found in any one perithecium was 60. Asci themselves were not found, so the explanation of this number of spores in a perithecium of a species which normally produces 8-spored asci is not immediately apparent. Two explanations are possible: (1) some spores in one or more asci failed to develop, or (2) the nuclear complement of a single ascus became divided among only four ascospores instead of among the usual eight. The occurrence of 4-spored asci was common in cultures of *Clathrospora diplospora*, Collection No. 33.

Perithecia about 250 μ in diameter; with fragile walls two or three cells thick; and with radiating, filamentous, pale yellow-brown, septate hyphae about 300 × 2.5 μ. These hyphae are attached to the wall cells of the perithecia and are distinct from the vegetative hyphae of indeterminate length and of lighter color. Ascospores 20.4–22.8 × 9.6 μ; septation 5–7 transverse with one series of longitudinal septa.

Cultures of Collection No. 5 of this species were treated in the same manner but did not produce perithecial initials.

PLEOSPORA NJEGUSENSIS Bub. (Collection No. 23)

Five subcultures of a single-spore isolate and 11 subcultures of a single-ascus isolate of this species produced mature perithecia on Leonian's agar in Petri dishes and in test tubes. The initial period at room temperature was 15 days. A large number of mature perithecia containing mature asci were produced within 16 weeks. The perithecia examined contained numerous asci with mature spores.

Perithecia mostly 500–800 μ in diameter, with heavy walls, and with a few hyphae originating in the wall cells of the upper part of the perithecium. These hyphae are not distinct in any character other than position from other hyphae present. Ascospores 28.8–46.8 × 13.2–21.1 μ; septation 7–9 transverse and two to three series longitudinal with additional longitudinal septa in individual cells.

PLEOSPORA TRICHOSTOMA (Fr.) Ces. & de Not. (Collection No. 29)

Two subcultures of single-ascus isolates and four subcultures of single-ascospore isolates of both Type I and Type II development produced perithecia on filter paper cones moistened with Leonian's medium. The initial period at room temperature was 10 days. Only a few large perithecia containing mature asci were produced within 32 weeks.

Similar isolates produced perithecia on Leonian's agar in test tubes. The initial period at room temperature was 10 days. Within 16 weeks large perithecial initials without ascus differentiation were observed. Within 20 weeks mature perithecia with numerous asci, a few of which contained mature ascospores, were produced.

Perithecia 200 μ to 1 mm in diameter, with heavy walls of opaque cells, and without distinctive hyphae originating in wall cells. Ascospores 45.6–50.4 × 18.0–19.2 μ; septation three transverse with one longitudinal septum in one or in each of both of the central cells.

CLATHROSPORA DIPLOSPORA (E. & E.) Wehm. (Collection No. 32)

Two subcultures of a gross isolate of ascospores from this specimen produced mature perithecia on Leonian's agar in test tubes. The initial period at room temperature was 14 days. Numerous small and a few relatively large perithecia containing many mature asci were produced by the end of 16 weeks.

Similarly treated cultures on Leonian's agar in Petri dishes produced well-defined perithecial initials, but asci were not found under these conditions within 32 weeks.

Perithecia 150–450 μ in diameter, with relatively fragile walls two or three cells in thickness, and mostly with numerous blunt or pointed, thick-walled, septate setae, but setae completely lacking on some perithecia. Ascospores 19.9–30.4 × 10.5–14.0 × 8.4 μ; septation three transverse with one longitudinal septum in one or in each of both of the central cells.

CLATHROSPORA DIPLOSPORA (E. & E.) Wehm. (Collection No. 33)

Twenty-one subcultures (Type I) derived from a single ascospore isolate of this species produced mature perithecia on Leonian's agar and on malt agar in test tubes. The initial period at room temperature was seven days. Numerous perithecial initials visible to the unaided eye were produced evenly scattered to crowded on and within the substrata within four weeks; perithecia containing mature ascospores were found in the same cultures only after about 16 weeks. Unruptured asci containing only four mature ascospores and without evidence of immature spores were not uncommon.

Similar cultures retained at room temperature under conditions which minimized loss of moisture did not produce perithecial initials within the space of eight weeks.

Three series of subcultures of this isolate on Leonian's agar were allowed different initial periods of growth at room temperature, namely, one day, seven days, and 14 days, after which they were removed to the cold room. Completely formed ascospores were found in cultures given an initial period of seven days fully a week before ascospores of similar maturity were found in cultures of either one day or 14 days of initial room-temperature growth.

Perithecia 175–380 μ in diameter; with relatively fragile walls of black-walled cells; and mostly with numerous simple, pointed, dark-walled, septate setae about 500–1500 μ in length and 3.5 μ in width at the base. These setae are quite distinct from other vegetative hyphae. Ascospores 24.6–43.3 × 15.2–24.6 × 14.4 μ; septation three transverse with one longitudinal septum in each of the two central cells.

Cultures of the type II isolate of this specimen were subjected to several different growth conditions, among which were the following:

1. Cultures initiated at room temperature and kept at room temperature under conditions which minimized loss of moisture; on Leonian's agar.

2. Cultures initiated at room temperature but removed to the cold room after seven days; (a) on Leonian's agar, (b) on a series of

Leonian's agars in which the nutrient contents were reduced to 5, 10, 25, and 50 percent of the standard amounts.

3. Cultures initiated at room temperature but removed to temperature of cold room after one, seven, and 14 days; Leonian's agar.

4. Cultures initiated at room temperature for one day; removed to a temperature below 0° C for periods of one, seven, and 14 days; then returned in part to a moist chamber at room temperature and in part to the cold room; filter paper cones moistened with Leonian's medium.

5. Cultures initiated at room temperature for two days; removed to temperature below 0° C for two days; then alternated between the two temperatures in 2-day periods for 14 days; finally placed in refrigerator room; filter paper cones moistened with Leonian's medium.

These cultures produced only conidial stages under all conditions imposed; perithecial initials were not observed at any time within test periods of nine to twelve months.

PLEOSPORA RAINIERENSIS Wehm. (Collection No. 37)

One subculture of a single-ascus isolate and four subcultures of single-ascospore isolates of this specimen produced perithecia on Leonian's agar in Petri dishes. The initial period at room temperature was 20 days. Only a few perithecia (two-four) were formed in any one culture; perithecia produced mature spores within 36 weeks but not within 32 weeks.

Perithecia 700 × 400 μ, with heavy walls, but without distinctive superficial hyphae. Ascospores 32.4–42.0 × 12.0–15.5 μ; septation seven transverse with one or two series of longitudinal septa and occasional additional longitudinal septa in individual cells.

C. *Discussion.*

The media used here as substrata for production of perithecia are not cited as being specific for perithecial formation by the species studied. Data presented indicate the probability that physical factors of the cultural conditions have positive influence upon the production of mature fruiting structures. Specifically, in this connection, must be mentioned the influence of low temperature, the initial period of growth at room temperature, and the time necessary for the maturation of cultures of the different isolates. A ready example illustrating the influence of these factors is found in the experiments with *Clathrospora diplospora* (Collection No. 33, Type I). Similar subcultures of the isolate were subjected to a low temperature and to room temperature during the

growth period; fruiting structures were produced at low temperature within four weeks but were not produced at room temperature within eight weeks. Other series of cultures allowed initial room-temperature growth periods of one, seven, and 14 days exhibited a maturation differential, in that mature perithecia appeared in cultures allowed seven days before mature structures appeared in the other cultures.

The length of time required for production and maturation of perithecia by isolates of the different species ranged from 16 to 36 weeks. *Pleospora njegusensis* and *Clathrospora diplospora* required only 16 weeks at low temperature for maturation. *P. trichostoma* required only 20 weeks when grown on Leonian's agar in test tubes but needed 32 weeks when grown on filter paper moistened with Leonian's medium. A length of time of about 36 weeks was required both by *P. ambigua* and by *P. rainierensis* before maturity was reached.

An explanation of the influence of these physical factors of low temperature, time, and initial treatment is not available from the data at hand. One possible explanation involves the probability that so-called staling products resulting from the physiologic activities of the mycelium collect in the medium or in the mycelium and, at a critical point in development, affect the initiation of fruiting. Another possibility rests on the fact that the rate of gaseous exchange in the medium is reduced at low temperatures, thus limiting the availability of atmospheric oxygen to the organism and possibly bringing about physiologic conditions in the mycelium that would favor perithecial formation. These suggestions and others of equally uncertain pertinence depend upon future investigation for clarification.

Judging from the results of these experiments, it is certain that the artificial conditions imposed upon the cultures did not prohibit the formation of perithecia by some of the isolates of the species used but, in fact, may have promoted such development. It is not certain, however, whether or not similar conditions actually inhibited the production of perithecia by isolates of other species cultured. There are several possible reasons for non-production of perithecia by the latter isolates: (1) five of the isolates were from single spores and presumably would not produce perithecia if the species they represent are heterothallic; (2) specific nutritional requirements of the isolates were not met by the substrata used; (3) specific environmental conditions necessary for perithecium production were not provided. It has been established that the length of time for which some cultures are allowed to remain at room temperature before being transferred to a low temperature has a positive effect upon the time of maturation of perithecial structures. Finally, it

has been demonstrated in the case of *Clathrospora diplospora* that a period of growth under low temperatures is necessary for the production of perithecial initials.

II. STATISTICAL ANALYSIS OF ASCOSPORE LENGTH

The determination of the range of size of ascospores has always been considered to be of primary importance in taxonomic circumscription of species of the Pleosporaceae. Usually the size-range indicated in the description of type material is accepted as the spore size-limit of the species, and only after studying numerous collections of what he considers to be one species does an investigator feel justified in emending a description. In practice, a worker, rather than change the established concept of a species, often announces new specific or varietal names for collections which vary from the type in ascospore dimensions, although other character differences may not be found. Such separation may or may not be valid, but the only certain way of finding out is by determining the variability in spore size of material of unquestionable species identity.

It cannot always be known with certainty that several similar specimens collected in different localities or on different substrata actually belong to the same species. For this reason any method of obtaining material from a single source sufficient for determining the variability of the organism under a large number of environmental conditions is desirable. The method most nearly meeting these requirements is that of pure-culture work with single-ascospore strains. By this means, provided the organism can be induced to fruit in culture, data can be obtained on a single organism under any desired number of conditions without fear of confusing characters of one fungus with those of another.

The following brief study is intended to show the variability of ascospore length within and between samples obtained from individual perithecia which were derived from a single ascospore or which were derived, in one case, from spores from a single perithecium.

A. *Materials and Methods.*

Isolates derived from ascospores of three different collections produced enough perithecia with sufficient numbers of mature ascospores that statistical methods of comparison could be used. The cultures of *Pleospora njegusensis* (Collection No. 23) and of *Clathrospora diplospora* (Collection No. 33) were derived from single ascospores; the culture of *C. diplospora* (Collection No. 32) was derived from at least two ascospores from a single perithecium. These cultures were maintained on

Leonian's agar under growth conditions listed previously and were sampled for the present purpose when numerous mature perithecia had been produced.

Each sample of ascospores was obtained by isolating and crushing a single mature perithecium in a drop of Amman's mounting fluid on a glass slide. By means of a mechanical stage the preparation was moved laterally across the field of the microscope and length data were recorded on all mature ascospores appearing in the field up to a total of 50 spores per perithecium. (A spore was considered to be mature if it had the minimum number of septa present which its generally accepted specific description indicated.) Data were recorded in terms of ocular micrometer divisions and statistical calculations were made on the data expressed in these units. Samples were obtained from four different perithecia of each of the isolates.

B. *Experimental Results.*

Making use of standard statistical formulae, the following values were determined from the length data of each sample: range of length, mean length, and standard deviation of mean length (TABLE I). The mean length values and the standard deviation of mean length values then were used in comparing data obtained from different samples by employing them in a standard ratio designed as a test for significance of difference between means (TABLE II).

C. *Discussion of Tables.*

Values derived from the data are arranged in the tables in such a manner that those values representing samples from individual perithecia may be read directly (TABLE I) and that those values representing comparisons of samples from different perithecia of the same culture may be found readily (TABLE II).[3]

The length-range data for samples from the different isolates (TABLE I) give a type of information usually found in published descriptions,

[3] In TABLES I and II the following symbols have been used in order to simplify and to standardize the method of reference to the individual perithecia and to the comparisons of different perithecia: *23, 32, 33* (cultures derived respectively from *Pleospora njegusensis, Clathrospora diplospora* of "normal" ascospore size, and *C. diplospora* of "large" ascospore size); 1, 2, 3, 4 (numbers assigned to individual perithecia studied); R (range of length in ocular micrometer divisions); Rμ (range of length in microns); m (mean length in ocular micrometer divisions); σ (standard deviation of length). In TABLE II values of *2.0* or more indicate that there exists a significant difference between mean length values of ascospore samples compared.

i.e., the observed limits of length of ascospores. For each isolate the length ranges of spores from the different perithecia are in close enough agreement that few investigators would consider it probable that any significant difference between samples actually exists. These length limits, however, do not reveal the pattern of variation in the different samples.

The mean length values (TABLE I) present information which offers a better means of comparison between samples. These mean values reveal that the patterns of variation in samples having identical size ranges are not necessarily the same. But conclusions concerning the significance of differences in mean length values of similar ascospore

TABLE I

RANGE OF LENGTH, MEAN LENGTH, AND STANDARD DEVIATION OF LENGTH OF ASCOSPORES FROM DIFFERENT SINGLE PERITHECIA IN CULTURES OF *Pleospora njegusensis* AND OF TWO STRAINS OF *Clathrospora diplospora*

Sample	R	Rμ	m	σ
23–1	27–40	31.59–46.80	32.90	3.47
–2	29–40	33.93–46.80	32.74	2.34
–3	27–40	31.59–46.80	31.18	2.61
–4	27–33	31.59–38.61	30.14	1.33
32–1	19–26	22.23–30.42	20.60	1.40
–2	18–23	21.06–26.91	19.96	1.23
–3	18–23	21.06–26.91	20.58	1.43
–4	17–23	19.89–26.91	20.16	1.28
33–1	21–37	24.57–43.29	30.20	2.79
–2	24–35	28.08–40.95	30.32	2.87
–3	25–37	29.25–43.29	29.50	2.89
–4	23–35	26.91–40.95	28.78	2.68

samples depend on more critical statistical comparisons. The results of such comparisons for the three isolates examined are recorded in TABLE II.

The most remarkable results of comparisons of ascospore samples from different perithecia of a single pure culture are apparent in the values listed for *Pleospora njegusensis* in TABLE II. In five of the six compared pairs of samples, significant differences in mean length are found between perithecia produced in a single culture of a single-spore isolate. The statistical interpretation of any one of these five comparisons is that, within the limits of random sampling, the chances are 19 to 1 against the mean length value of one sample being obtained from a random sample of spores from the second perithecium. This means, in application, that it probably would be possible to examine a relatively large number

of perithecia of *P. njegusensis* and find numerous different patterns of variation in ascospore size. If such a variety of spore-length patterns can be found in one pure culture derived from a single ascospore, it seems reasonable that a worker could expect to find even greater ranges of spore variation and more numerous different length patterns in material grown on different artificial media or collected in nature. The specific circumscription of this organism probably must be extended eventually to include diverse growth forms now excluded by its original description.

The comparisons of data from samples of the two different isolates of *Clathrospora diplospora* yield values which indicate that, for the most part, these isolates vary within their own well-defined limits and in a relatively small number of length variation patterns under the experi-

TABLE II

DEGREE OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN MEAN LENGTHS OF ASCOSPORES FROM DIFFERENT SINGLE PERITHECIA IN CULTURES OF *Pleospora njegusensis* AND OF TWO STRAINS OF *Clathrospora diplospora*

Perithecium numbers	*P. njegusensis*	*C. diplospora* Coll. No. 32	*C. diplospora* Coll. No. 33
1–2	0.3	*2.5*	0.2
1–3	*2.8*	0.1	1.2
1–4	*5.3*	1.6	*2.6*
2–3	*3.2*	*2.3*	1.4
2–4	*6.8*	0.8	*2.8*
3–4	*2.5*	1.6	1.3

mental conditions employed. In one-third of the comparisons for each of these isolates significant mean differences between samples from different perithecia were found. It would be expected that more pronounced variation would be found in material from different substrata. However, judging from the length ranges and the mean-length values of these two different isolates, it appears highly improbable that they are genetically the same. A comparison of ascospore size between samples from perithecia in the original collections and from perithecia produced in culture is indicated here:

Collection No. 32
in nature: 25.7–26.9 × 12.9–15.2 × 8.2–9.2 μ
in culture: 19.9–30.4 × 10.5–14.0 × 8.2 μ
Collection No. 33
in nature: 31.6–37.4 × 16.4–18.7 × 10.5–12.9 μ
in culture: 24.6–43.3 × 15.2–24.6 × 14.4 μ

Although the shape and septation of ascospores of the two fungi are the same, the size ranges of the spores from nature do not overlap and the size ranges of spores from cultures do not overlap significantly. Examination of perithecial material obtained on different substrata may necessitate extension of the spore-length circumscription of the species as described, but it is considered unlikely that such an extension based on pure cultures of the small-spored isolate would include the spore range of the large-spored isolate. On the basis of the cultural and statistical data presented here, the two "strains" of *C. diplospora* studied must be considered as separate species.

GENERAL DISCUSSION

The present investigation has shown that experimental studies of the inherent variability of species in the Pleosporaceae must be accomplished before accurate species circumscriptions can be established. For this reason the observations herein noted place emphasis upon a better understanding of the individual species rather than upon their identification.

The present state of affairs in the systematics of *Pleospora* and *Clathrospora* is outlined and enlarged upon by Wehmeyer (**1946**, **1948**, and **1949–1953**) in his studies of these genera and of the closely related genus *Leptosphaeria*. From his observations on numerous collections of *Pleospora*, it is his opinion (**1948**) that, "although occasional collections or groups of collections stand out as distinct, the great majority fall into larger groupings showing . . . overlapping variations . . . for any one of a number of important characters." He maintains that the septation of mature ascospores is the least variable character of species in this group of fungi and uses this character, in correlation with ascospore form, size, and color and with data on perithecia, asci, and habitat, in delimiting species or species-complexes. Because of the high degree of intergradation in characters of different collections, Wehmeyer has concluded that the best way at present of handling series of *Pleospora* forms which are morphologically intergradient is to set certain limits of variation more or less arbitrarily and to refer to each group either by a commonly accepted binomial or as a "species-complex" (e.g., *Pleospora vagans* complex). He admits frankly that such separations are conservative and that "further study of larger numbers of collections may show other characters which may be used to segregate smaller groups as species or varieties, or may advise the union of groups now supposed to be good species" (**1946**).

It is interesting but not surprising that statistical work described here for lengths of ascospores from pure cultures bears out rather well Weh-

meyer's conservative taxonomic procedure based completely on material collected from natural habitats. The statistical results recorded for one subculture of a single-spore isolate of *Pleospora njegusensis*, for instance, indicate that ascospores of this species vary in numerous different length-patterns, and it is conceivable that, had a collector found any two different patterns in nature or had they been described individually from nature, two different "varieties" or even "species" might have been proposed. With the knowledge that the spores of *P. njegusensis* are significantly variable, even in pure culture, it would seem ill-advised to consider describing the morphologic variants of the species as entities at any taxonomic level. This conclusion may be considered as strengthening the view of Wehmeyer that many collections of *Pleospora* with intergrading morphology are best handled at present in relatively large taxonomic blocks.

The statistical results recorded for the isolates of the two different spore-forms of *Clathrospora diplospora* might be considered as supporting Wehmeyer's view that "occasional collections or groups of collections stand out as distinct" (**1948**), since within the culture examined for each of these isolates the variation in length patterns of ascospores is decidedly limited. In addition, the size ranges of ascospores of the two fungi in nature have not been observed to overlap, and the size ranges of spores from culture do not overlap significantly. For these reasons the isolates studied are considered to represent two distinct species of *Clathrospora*.

Conclusions drawn from the statistical data in conjunction with the facts known about the origins of the isolates might appear to be contradictory when results of the conidial work (Simmons, **1952**) and of the present ascospore work are compared. These conclusions assert, on the basis of similar types of observations, that in one instance the form-species of *Alternaria* may contain several different organisms, whereas in the other instance a species circumscription in *Pleospora* or in *Clathrospora* may represent only a portion of the variation potential of one organism. These data and conclusions, however, actually reflect the present-day situation in the taxonomy of the two groups, i.e., individual form-species of some Fungi Imperfecti have been used as receptacles for morphologically similar but genetically distinct organisms whereas, in the Pleosporaceae, several portions of the natural population of a single genetic entity may have been circumscribed as different species without an understanding of the inherent variability of the organism.

It is interesting to speculate on the possibility of setting up a system by which the variability of a specific organism could be determined and,

in consequence, by which its identity in a natural system of classification could be defined. It is believed that such an ideal method cannot be accomplished alone by examination of material discovered in nature; the unknown factors of environmental influence are too numerous. On the other hand, it is not maintained that culture work under controlled but artificial conditions can yield the final answer. A plan aimed at a realistic investigation of a species must incorporate observations both from nature and from culture with the foundations of the system being based on experimental procedures which can be defined and controlled. More specifically, the type of variation usually discussed by taxonomists in connection with the characters of a natural population cannot be admitted in an attempt to establish a sound basis for species circumscription, since such variation in a "species" is assumed on the basis of similarity in morphology of different portions of the population. It is suggested that variation established in cultures of an organism derived from a known source may be used as a basis for interpreting the variation in populations found occurring naturally.

SUMMARY

1. Statistical analyses of ascospore lengths in samples derived from different perithecia of a single culture each of *Pleospora njegusensis*, of *Clathrospora diplospora* ("normal" ascospore size), and of a species referred to as a "large-spored variety" of *C. diplospora* indicated that ascospores of *P. njegusensis* vary in numerous different patterns of length and that ascospores of each of the isolates of "*C. diplospora*" vary in a decidedly smaller number of different length-patterns.

2. Ascospore-size data and statistical data on ascospore length indicated that the two fungi referred to as different strains of *Clathrospora diplospora* are two distinct species of *Clathrospora*.

3. Production of mature perithecia by different strains of *Pleospora* and *Clathrospora* appeared to be positively influenced by the physical factors of room temperature during the initial period of growth, of low temperature during the remainder of the growth period, and of the total growth time.

4. The number of mature perithecia produced by individual isolates on Leonian's agar often was characteristic for a species.

5. The cultural characters of separate isolates of single species often were different:

a. *Pleospora ambigua:* one isolate produced mature perithecia in culture; two other isolates did not.

b. *Pleospora njegusensis:* the isolate from ascospores of one collection produced mature perithecia in culture; a similar isolate from a second collection did not.

c. *Pleospora trichostoma:* two distinct growth types were produced by different single-spore strains; however, no differences in the type or quantity of perithecial production were found in any of the cultures.

d. *Clathrospora diplospora:* strains derived from ascospores from two different collections produced mature perithecia in culture; a third strain derived from ascospores from the same collection as one of the perithecial strains produced only conidia in culture.

The author welcomes this opportunity to express his continuing gratitude to Dr. L. E. Wehmeyer for guidance throughout the course of these studies and to Dr. A. H. Smith for editorial criticism of the manuscript.

LITERATURE CITED

Bauke, H. 1877. Zur Entwickelungsgeschichte der Ascomyceten. Bot. Zeit. **35**: 313–326.

Brefeld, O. 1891. "Pleosporeen," in Untersuchungen aus dem Gesammtgebiete der Mykologie. Münster i. W.:Heinrich Schöningh. Part X (Ascomyceten II), pp. 218–228.

Cavara, F. and N. Mollica. 1907. Ricerche intorno al ciclo evolutivo di una interessante forma di *Pleospora herbarum* (Pers.) Rab. Ann. Myc. **5**: 119–149.

Cocchi, F. 1931. Un marciume dei limoni dovuto a *Pleospora herbarum* (Pers.) Rabenh. Boll. R. Staz. Pat. Veg. (Rome), n.s. **11**: 179–213.

Diedicke, H. 1902. Ueber den Zusammenhang zwischen *Pleospora-* und *Helminthosporium*-Arten. Centr. Bakt. Parasit. und Infektionskr. **9**: 317–329.

——. 1903. Ueber den Zusammenhang zwischen *Pleospora-* und *Helminthosporium*-Arten. II. Centr. Bakt. Parasit. und Infektionskr. **11**: 52–59.

Drechsler, C. 1923. Some graminicolous species of *Helminthosporium:* I. Jour. Agr. Res. **24**: 641–739.

Gibelli, G. and L. Griffini. 1874. Sul polimorfisma della *Pleospora herbarum* Tul. Arch. Trien. Lab. Bot. Critt. (Pavia) **1**: 53–92.

Groves, J. W. and A. J. Skolko. 1944. Notes on seed-borne fungi. I. *Stemphylium.* Can. Jour. Res. **22**: 190–199.

Halsted, B. D. 1893. *Pleospora* of *Tropaeolum Majus.* N. J. Agr. Exp. Sta. Rept. (1892) **13**: 290–293.

Hughes, S. J. 1945. A leaf-spot of mangolds caused by *Pleospora herbarum* (Pers.) Rabenh. Trans. Brit. Myc. Soc. **28**: 91–93.

Klebahn, H. 1918. Haupt- und Nebenfruchtformen der Askomyzeten. Leipzig: Gebrüder Borntraeger. Teil I. 395 pp.

Kohl, F. G. 1883. Ueber den Polymorphismus von *Pleospora herbarum* Tul. Bot. Centr. **16**: 26–31.

Leonian, L. H. 1924. A study of factors promoting pycnidium-formation in some Sphaeropsidales. Am. Jour. Bot. **11**: 19–50.

Miyabe, K. 1889. On the life history of *Macrosporium parasiticum* Thüm. Ann. Bot. **3**: 1–24.

Noack, F. 1905. *Helminthosporium gramineum* Rabenh. und *Pleospora trichostoma* Wint. Zeitschr. Pflanzenkr. **15**: 193–205.

Simmons, E. G. 1952. Culture studies in the genera *Pleospora, Clathrospora,* and *Leptosphaeria.* Mycologia **44**: 330–365.

Wehmeyer, L. E. 1946. Studies on some fungi of northwestern Wyoming. III. *Pleospora* and *Leptosphaeria.* Lloydia **9**: 203–240.

——. 1948. The developmental pattern within the genus *Pleospora* Rab. Mycologia **40**: 269–294.

——. 1949–1953. Studies in the genus *Pleospora.* I. Mycologia **41**: 565–593. 1949. II. Lloydia **15**: 65–109. 1952. III. Mycologia **43**: 34–53. 1951. IV. Mycologia **43**: 570–589. 1951. V. Mycologia **45**: 391–414. 1953. VI. Am. Jour. Bot. **39**: 237–243. 1952.

SEXUAL REPRODUCTION IN GONAPODYA [1]

ROBERT M. JOHNS [2] AND R. K. BENJAMIN [3]

(WITH 17 FIGURES)

The affinities of the genus *Gonapodya* in the aquatic Phycomycetes have been a point of conjecture since its initial description. It has been placed in the families Monoblepharidaceae (**1**, **2**), Saprolegniaceae (**6**), Leptomitaceae (**8**), and the Blastocladiaceae (**5**). These diverse opinions as to the taxonomic position of *Gonapodya* may be attributed to its rather infrequent collection and to previous failure to demonstrate conclusively a sexual mechanism. The only previous accounts of supposed sexual structures in the genus are those of Cornu (**3**), who reported antherozoids and terminal oospores of *G. prolifera* similar to those of *Monoblepharis,* and Thaxter (**8**), who described and figured detached oogonia with applied antheridia which he found associated with thalli of *G. polymorpha.* Neither of these reports was confirmed by subsequent investigations.

In the spring of 1952, collections made by the authors in North Carolina and Massachusetts provided generous growths of water molds referable to the genus *Gonapodya.* Abundant growth of *G. polymorpha* was obtained on various types of hardwood twigs from several streams and ponds near Chapel Hill, North Carolina. Oak twigs collected in a small pond near Winchester, Massachusetts, yielded quantities of both *G. prolifera* and *G. polymorpha.* Examination of these collections led to the observation of a phenomenon which could be interpreted only as a type of sexual reproduction, one which parallels closely that described for *Monoblepharella* (**7**).

[1] The sexual phase of *Gonapodya* was observed and studied independently by each of the authors. The content of this paper represents a combination of these independent observations.

[2] This work was done in partial fulfillment of requirements for the degree of Master of Arts in Botany at the University of North Carolina, and it was directed by Dr. John N. Couch, to whom the author wishes to express his sincere gratitude.

[3] The writer wishes to thank Dr. Wm. H. Weston, Jr., for his encouragement during the progress of this study which was carried out during the tenure of the author as a National Research Fellow in Botany, The Biological Laboratories, Harvard University, 1951–52.

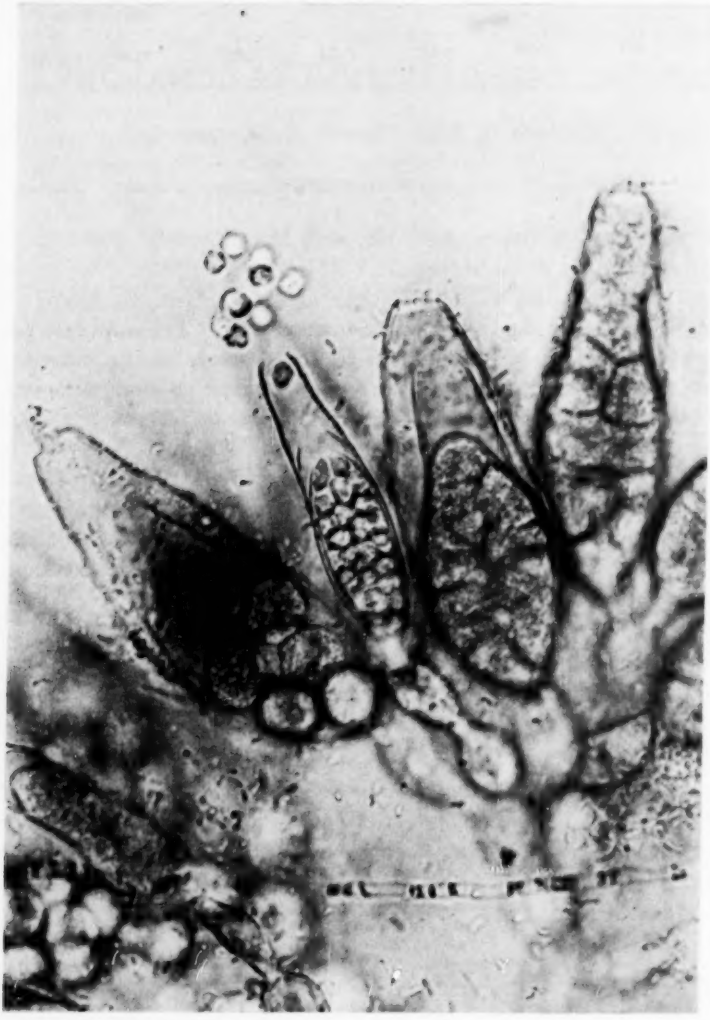


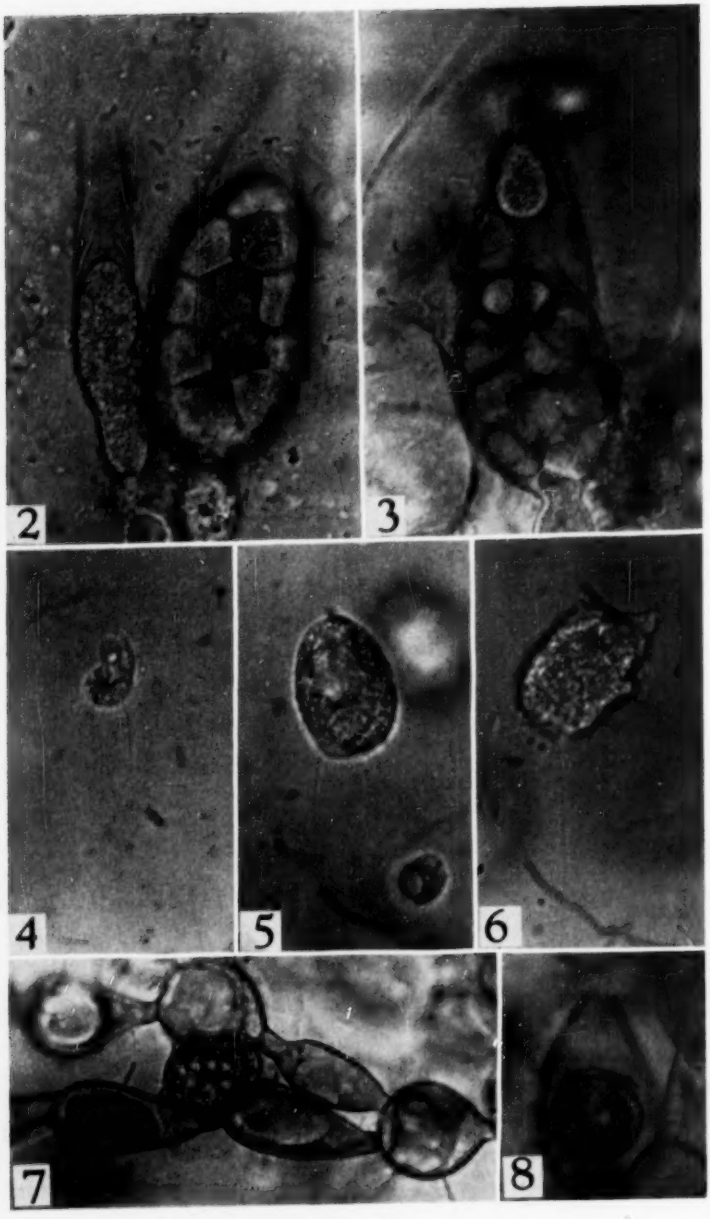
FIG. 1. Gametangia of *Gonapodya prolifera*. At the center a discharging male gametangium showing several recently discharged antherozoids, flanked on the left by a female gametangium with several mature eggs and on the right by two female gametangia with eggs in earlier stages of differentiation, × 680.

Sexual reproduction in *Gonapodya* is effected by posteriorly uniflagellate male gametes and non-flagellate female gametes borne in gametangia formed terminally on the thallus branches. Both male and female gametangia are capable of renewal by internal proliferation. The small male gametes or antherozoids, which contain a conspicuous apical cluster of refractive granules, are discharged from the gametangia as in *Monoblepharis* and *Monoblepharella*. The female gametes or eggs are distinguished by their lack of flagella, relatively large size, and possession of large numbers of minute refractive granules which impart to them a characteristic brown color. The eggs may or may not be discharged from the female gametangium, and, consequently, fusion of the egg and antherozoid may occur either inside or outside of the female gametangium. In either case fusion is incomplete, a portion of the body of the male gamete with its flagellum remaining evident upon the surface of the egg. The resulting fusion cell, after a variable period of amoeboid movements, assumes an ovoid shape and enters upon a motile stage during which it is propelled by the male flagellum as in *Monoblepharella*. Following the motile stage, which has been observed to last as long as three hours, the fusion cell comes to rest, assumes a spherical shape, and develops into a smooth-walled, brownish oospore. Zygote germination has not been observed.

Although the sexual phases of both the described species of *Gonapodya* follow the basic pattern described above, there are quite distinctive differences in the size and shape of the sexual structures formed by *G. prolifera* and *G. polymorpha*. In view of these distinctions and of the general paucity of morphological data concerning these fungi, concise descriptions of the sexual phase of each are here included.

GONAPODYA PROLIFERA (Cornu) Fischer (4)

Mycelium composed of hyphae which are more or less regularly divided by pseudosepta into short-elliptical to long-clavate segments, successively subumbellately branched, the branches diverging in a compact to more or less open tuft from a common base. Male and female gametangia once to at least five times proliferous; successive gametangia progressively smaller, borne singly or in small groups on the terminal cell of a branch and separated from it by clearly defined constrictions. Female gametangia elongate-ovoid, inflated below, tapering abruptly or gradually to a blunt apex, forming a single discharge papilla, 60–180 × 25–35 μ; female gametes variable in number, up to 20 in a gametangium, globose, non-flagellate, 15–19 μ in diameter. Male gametangia smaller and more slender than the female, forming a single discharge papilla,



FIGS. 2–8.

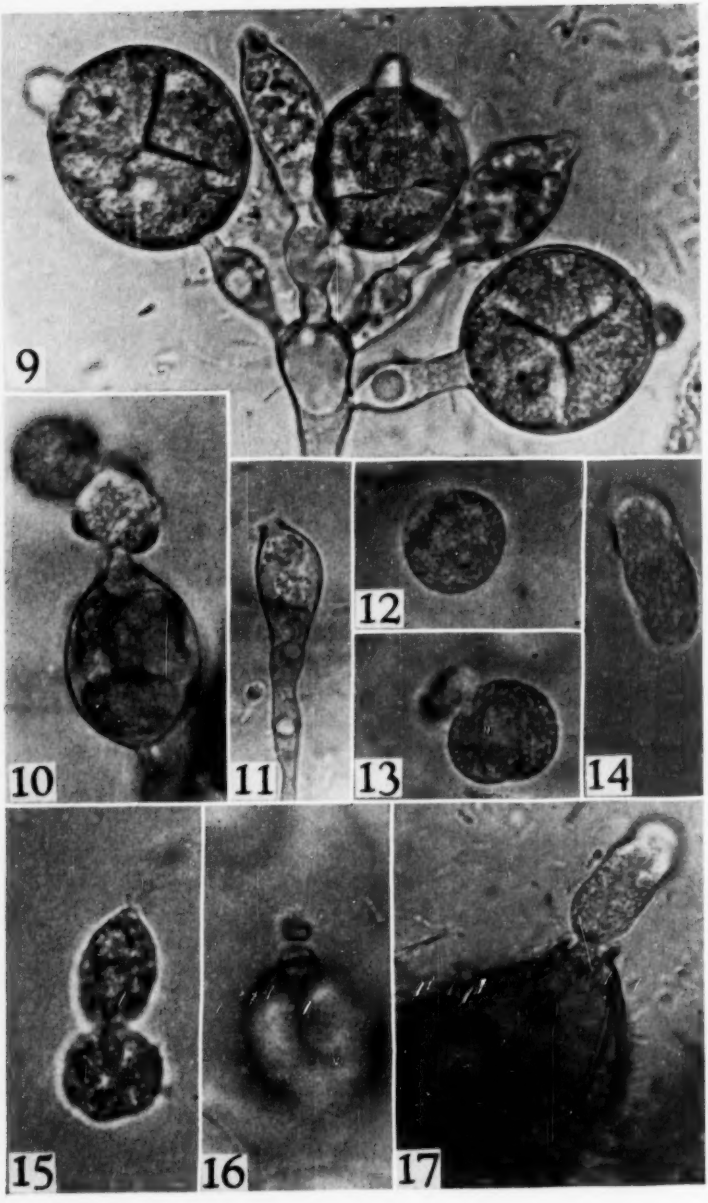
40–107 × 10–15 μ; male gametes variable in number, up to 40 in a gametangium, posterioly uniflagellate, elliptical or somewhat cylindrical, with a conspicuous anterior cluster of refractive granules, 7–10 × 3–6 μ, flagellum 22–24 μ in length. Zygote retaining the flagellum of the male gamete, at first amoeboid and with varying shape, characteristically with a hyaline anterior pseudopodium; finally actively motile, posteriorly uniflagellate, ovoid, often reverting to the amoeboid condition, 20–25 × 11–15 μ; encysting to form a smooth-walled, spherical resting spore surrounded by a hyaline envelope; germination not observed.

GONAPODYA POLYMORPHA Thaxter (8)

Mycelium more or less irregularly or dichotomously branched, consisting usually of long, slender, irregular hyphae in which the segmentation is more or less indistinct or obsolete but with occasional internal thickenings or pseudosepta; bearing terminally one or more subumbellate tufts or short-segmented branchlets. Male and female gametangia terminal and solitary or several arising from a single segment, up to three times proliferous, hyphae sometimes growing through and beyond discharged gametangia. Female gametangia subspherical to short-ovoid, forming 1–4 discharge papillae, 20–60 × 15–25 μ; female gametes variable in number, up to 16 (usually 6–8) in a gametangium, spherical, non-flagellate, 11–18 μ in diameter. Male gametangia smaller than the female, short-elliptical to ovoid, forming a single, terminal discharge papilla, 20–25 × 10–15 μ; male gametes variable in number, up to 18 in a gametangium, posteriorly uniflagellate, elliptical or somewhat cylindrical, with a conspicuous anterior cluster of refractive granules, 7–9 × 3–5 μ, flagellum 21–23 μ in length. Zygote retaining the flagellum of the male gamete, at first amoeboid, then actively motile, posteriorly uniflagellate, ovoid, 16–25 × 10–15 μ; encysting to form a smooth-walled, spherical resting spore; germination not observed.

Several significant differences in sexual structures emphasize the distinctness of these two species. There is a marked difference in size;

FIGS. 2–8. *Gonapodya prolifera*. 2. Male and female gametangia. The male (left) has not yet begun to differentiate gametes. The female (right) contains eggs in an advanced stage of cleavage; note the conspicuous nuclear area, × 700. 3. Female gametangium containing mature eggs one of which is in the process of being discharged, × 700. 4. Flagellated antherozoid. Photographed while creeping about on the undersurface of the coverglass, × 1000. 5. Zygote shortly after fusion of egg and antherozoid. The flagellum and posterior surface of the male gamete may be seen projecting from the zygote (lower left). An antherozoid with its flagellum projecting upward toward the zygote is at the lower right, × 1000. 6. An amoeboid zygote. Note the anterior pseudopodia and the posterior flagellum, × 1000. 7. Encysted zygote attached to the hyphae by the hyaline cyst wall, × 820. 8. Zygote which has encysted inside a female gametangium, × 820.



FIGS. 9–17.

in reference to the various structures associated with sexual reproduction as well as with the general habit of the thallus, *Gonapodya polymorpha* is a much smaller and more delicate organism than *G. prolifera*. The female gametangia of *G. prolifera* are elongate-ovoid (siliquaeform); those of *G. polymorpha* are characteristically sub-spherical. It appears characteristic for the female gametangia of *G. polymorpha* to possess more than one papilla.

The striking similarity of sexual reproduction in *Gonapodya* to that previously described for members of the Monoblepharidales is convincing evidence of its close relationship to this group. The Monoblepharidales is characterized by the possession of a type of sexual reproduction which is unique among the fungi: the fertilization of non-flagellate eggs by motile, posteriorly uniflagellate antherozoids. It is evident that *Gonapodya* is within the limits of this definition. Similarities in the appearance and behavior of the gametes of *Gonapodya* and of *Monoblepharis* and *Monoblepharella* also merit consideration. Perhaps most striking is the existence of a motile stage of the fusion cell of *Monoblepharella* (**7**) which is identical in all basic respects with that described here for *Gonapodya*. The inclusion of this genus in the order Monoblepharidales seems certainly justified by these new data.

SUMMARY

Sexual reproduction is reported for *Gonapodya*, a genus of the aquatic Phycomycetes. This sexual process is characterized by the fertilization of a relatively large non-flagellate egg by a small, posteriorly uniflagellate antherozoid. The fusion is for a time incomplete, the zygote becoming actively motile, propelled by the male flagellum. Encystment follows the motile stage, resulting in the formation of a smooth-walled spherical oospore.

Description of the sexual phase of each of the two species of *Gonapodya*, *G. prolifera* and *G. polymorpha*, are given.

FIGS. 9–17. *Gonapodya polymorpha*. 9. A fascicle of three subspherical female gametangia and two ovoid male gametangia with gametes in late stages of differentiation, × 1100. 10. Discharge of eggs from a female gametangium, × 1070. 11. Male gametangium containing three antherozoids, × 875. 12. Mature egg, × 1100. 13. Antherozoid creeping about on the surface of an egg, × 1100. 14. Amoeboid zygote, × 950. 15. Two swimming zygotes. The one above shows the ovoid shape characteristic of the active zygote, × 820. 16. An antherozoid entering a female gametangium by way of the discharge papilla of the latter, × 1050. 17. Zygote emerging from a female gametangium following fusion of gametes inside, × 950.

The relationship of *Gonapodya* to established genera of the order Monoblepharidales, especially *Monoblepharella,* is indicated, and, it is believed, justifies the inclusion of *Gonapodya* in that order.

DEPARTMENT OF BOTANY,
UNIVERSITY OF MICHIGAN,
ANN ARBOR, MICHIGAN
AND
RANCHO SANTA ANA BOTANIC GARDEN,
CLAREMONT, CALIFORNIA

LITERATURE CITED

1. **Cornu, M.** 1871. Note sur deux genres nouveaux de la famille des Saprolégniées. Bull. Soc. Bot. France **18**: 58–59.
2. ——. 1872. Monographie des Saprolégniées; étude physiologique et systématique. Ann. Sci. Nat. Bot. 5 sér., **15**: 1–198, pls. 1–7.
3. ——. 1877. Remarques sur quelques Saprolégniées nouvelles. Bull. Soc. Bot. France **24**: 226–228.
4. **Fischer, E.** 1892. Phycomycetes. Die Pilze Deutschlands, Oesterreichs und der Schweiz. Rabenhorst, Kryptogamen-Fl. **1(4)**: 1–490.
5. **Fitzpatrick, H. M.** 1930. The lower fungi. Phycomycetes. McGraw-Hill Book Company, Inc., New York.
6. **Reinsch, P. F.** 1878. Beobachtungen über einige neue Saprolegnieae, über die Parasiten in Desmidienzellen, und über die Stachelkugeln in Achlyaschlaüchen. Jahrb. wiss. Bot. **11**: 283–311, pls. 14–17. 1877.
7. **Sparrow, F. K.** 1940. Phycomycetes recovered from soil samples collected by W. R. Taylor on the Allen Hancock 1939 Expedition. Allen Hancock Pacific Expeditions. Publ. Univ. So. Calif. Press **3(6)**: 101–112, pls. 16–17.
8. **Thaxter, R.** 1895. New or peculiar aquatic fungi. 2. *Gonapodya* Fischer and *Myrioblepharis* nov. gen. Bot. Gaz. **20**: 477–485, pl. 31.

TAXONOMIC STUDIES IN THE GENUS COCCOSPORA

SAMUEL C. DAMON AND MARY H. DOWNING

(WITH 2 FIGURES)

The validity of the genus *Coccospora* Wallroth (**1833**) has been under question (Shear, **1939**; Gilman, **1945**) for some time. Since its establishment in the Moniliales on the type *C. aurantiaca*, eight little-known species have been added to the genus. Examination of type material shows *Coccospora aurantiaca* to be sclerotial in structure and suggests more suitable genera in which to distribute the later non-sclerotial species which were placed in the genus. The present study is not meant to be broad enough to indicate the specific position of the type species in the genus *Sclerotium*, although recommendations concerning the disposition of other species are made.

1. COCCOSPORA AURANTIACA Wallroth. 1833. Flora Crypt. Germ. **2**: 176.
   Sphaerosporium lignatile Schw. 1832. Trans. Am. Phil. Soc. n.s. **4**: 303.
   Myxosporium aurantiacum Rabh. 1844. Rabh. Krypt. Fl. I. Aufl. **1**: 41.
   Protomyces xylogenus Sacc. 1879. Michelia **1**: 14.
   Allescheriella uredinoides P. Henn. 1897. Hedwigia **36**: 244.
   Bactridiopsis ulei P. Henn. 1904. Hedwigia **43**: 397.

Wallroth's original description of *Coccospora* noted large, translucent, globose spores in a distinct membrane, held together in loose, slimy, amorphous, golden masses easily dissolved in water. The type species, *C. aurantiaca* (the only species described at the time), was found on decaying wood in Thuringia.

In the present study, re-examination of the type specimen disclosed small fragments of sclerotial material, apricot color in mass, containing irregularly-shaped, thick-walled cells, brilliant golden-yellow in transmitted light, and measuring 21–33 μ in diameter (FIG. 1, A, B). Although identity is not implied, a comparison of this illustration with that of sclerotia of *Penicillium* (Raper and Thom, p. 53) shows a strong

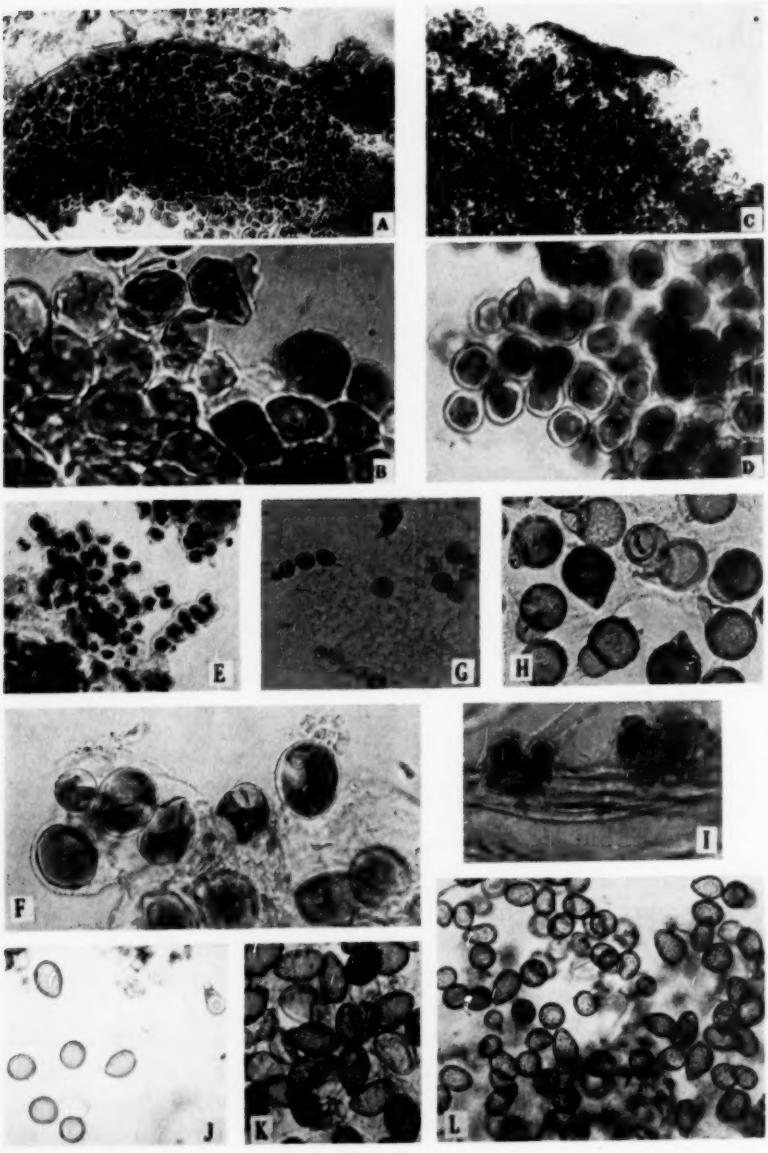


FIG. 1.

similarity of structural detail. Of specimens examined, this material most nearly resembles the type of *Sclerotium applanatum* Schw. (FIG. 1, C, D), which also has irregularly-shaped, more or less globose, rough, thick-walled cells, but which differs in having them arranged in a more nearly linear fashion. Although this evidence indicates that *C. aurantiaca* properly belongs in *Sclerotium*, no satisfactory relocation of the species can be made without a comprehensive study of the latter genus.

2. COCCOSPORA MUSCORUM Karst. 1884. Hedwigia **23**: 88.
Protomyces muscorum Karst. 1884. Hedwigia **23**: 40.

Described as forming round to variform, rust-colored acervuli which may become confluent, and are 0.5–1 mm broad; spores globose, 30–55 μ in diameter, golden-yellow, enveloped in slime, usually with many massed together.

Habitat: on moss (*Hypna*), Finland.

This organism was first described under the name *Protomyces muscorum* Karst., and it is listed as such by Saccardo (**1888**) in the Sylloge Fungorum **7**: 321; and by Oudemans (**1919**) in Enumeratio Systematica Fungorum **1**: 273. The latter treats *Coccomyces muscorum* Karst. as a synonym. The type specimen bears the label *Protomyces muscorum* Karst., but the *Protomyces* has been crossed out and the name *Coccospora* written over it in the same handwriting, presumably Karsten's. It contains a moss, one small section of which is covered with masses of brilliant yellow-orange, glistening, hyaline cells which, under low magnification, are similar to the spores of *Sphaerosporium lignatile* Schw. However, a mount shows masses of globose to subglobose, hyaline to golden-brown, smooth-walled cells enclosed in a hyaline membrane. The cells (FIG. 1, E, F) measure 39.2–47.6 × 38–44.8 μ, with a wall 1.4 μ thick, and contain a cytoplasm that stains deeply, showing filaments and some spindle-shaped bodies. A mucoid membrane, greatly varying in thickness (2.8–12.6 μ broad), surrounds each cell, either individually or in large masses. Occasionally a small amount of thin (1.4 μ thick) mycelium can be seen around the outside

FIG. 1. A, B. *Coccospora aurantiaca* Wallr. TYPE, A × 130, B × 560; C, D. *Sclerotium applanatum* Schw. TYPE, C × 130, D × 560; E, F. *C. muscorum* Karst. TYPE, E × 58, F × 250; G. *C. casei* Karst. TYPE, × 250; H. *C. rosea* Karst. TYPE, × 250; I. *C. rubiginosa* Speg. TYPE, × 250; J, K. *C. sinensis* Teng: J, Bureau of Plant Industry collection, × 250, K, Cornell University Herbarium collection swollen in 18% KOH and stained with phloxine, × 250; L. *Allescheriella crocea* (Mont.) Hughes mounted in lacto-phenol and stained with cotton blue, × 250. A–J mounted in Hoyer's solution and stained with acid fuchsin.

of the mucoid mass. The cells are first produced within the leaf of the moss and are finally erumpent.

After examining a slide of the material, Dr. George W. Fischer [1] suggests that this organism might possibly be a chytrid. Dr. John S. Karling concurs in this suggestion, stating [1] that it may be a species of *Synchytrium* or of *Physoderma* but that such a determination is difficult to ascertain from preserved material.

It is believed that this material is not hyphomycetous in nature and is definitely not related to the type of *Coccospora*.

3. COCCOSPORA CASEI Karst. 1890. Rev. Mycol. **12**: 80.

The original article describes an orbicular, but not confluent, orange-red, loose turf; conidia globose, 9–12 μ in diameter, light golden-orange, with hyaline membrane; hyphae scarce, ramose, up to 70 μ long, 3 μ thick.

Originally found on cheese in Finland.

The present examination of Karsten's type specimen shows the surface of the cheese to be covered with globular, yellow-orange, glistening masses of spores which have taken up some of the oil of the cheese. Individual spores are not distinguishable under stereoptican microscopic power. Microscopic examination shows spores globose to subglobose, hyaline, thick-walled, definitely flattened at one end, borne in chains, and measuring 7.8–15.6 × 7.8–14.3 μ, with walls 1.4–2.1 μ thick (FIG. 1, G). Except in spore size, this specimen appears to be more closely related to *Sphaerosporium lignatile* Schw. than to the type of the genus *Coccospora*.

4. COCCOSPORA PARASITICA Saccardo, Bommer & Rousseau. 1890. In Bommer & Rousseau, Bull. Soc. Roy. Bot. Belg. **29**: 295.

The original macroscopic characters were listed as granular, waxy, transparent, white, becoming dull yellow, densely agglomerated. Conidia hyaline, globose, at first naked, then with very thick membrane, smooth, 15–21 μ.

On old stromata of *Pleomassaria siparia, Coryneum notarisianum, Eutypella stellulata*, etc., Belgium.

The type specimen has not been available for study, and no illustrations were given by Bommer and Rousseau.

5. COCCOSPORA ROSEA Karst. 1892. Hedwigia **31**: 296.

Originally described as follows: Turf effuse, waxy, reddish; hyphae short, branching, jointed and knotty, hyaline, 6–10 μ thick, with a sub-

[1] Personal communication, 1952.

globose knotty surface, very extensive; conidia globose, thick-walled, hyaline to pale yellow, rather tough, 27–30 μ in diameter.

Habitat: on decaying *Tricholoma saponaceum* Fr. at Mustiala, Finland.

Examination of the type specimen reveals clusters of cinnamon-brown to reddish, glistening spores growing over most of the insect-ravaged agaric and spreading over the moss which is attached to the specimen. Mounts from these colonies show the typical, two-celled spores of *Mycogone rosea* Link, with the very rough terminal cell measuring 26–33.8 × 26–28.6 μ and the lower somewhat smoother cell measuring 13–18.2 × 10.4–13 μ (FIG. 1, H). This character must have been overlooked by Karsten. It is obvious that *Coccospora rosea* Karst. must be referred to the synonymy of *Mycogone rosea* Link.

6. COCCOSPORA RUBIGINOSA Speg. 1910. Anal. Mus. Nac. Buenos Aires. Series 3. **13**: 413.

Described as having acervuli, at first hemispherical, 250–500 μ in diameter, commonly discrete, then confluent in subgelatinous honey-colored liquid; hyphae thin, hyaline, short and soon branching; conidia 20–30 μ in diameter, thickly gathered into a ball and deliquescing on connecting hyphae.

Habitat: on trunk of *Passiflora coerulea*, Tucuman, Argentina, 1905.

The type specimen has been badly damaged by insects, and Prof. Lindquist informs us that an examination made by Prof. Marchionatto revealed only a *Verticillium* on the material. We have seen this but have also noted a very occasional cluster of globose, reddish-brown spores, measuring 15.6–19.5 μ, with a wall 1.4 μ thick, and enveloped in a mucous membrane (FIG. 1, I), much in the manner of the spores in the type of *C. muscorum*. No indication of how the spores were borne could be found. No illustrations were given in the original diagnosis. It is difficult to suggest a disposition of this species on such limited material.

7. COCCOSPORA AGRICOLA Goddard. 1913. Bot. Gaz. **56**: 265.

Recently reduced to synonymy under *Botryotrichum piluliferum* Sacc. and March. by Downing (**1953**) and previously given consideration by White and Downing (**1951**).

8. COCCOSPORA SINENSIS Teng. 1936. Sinensia **7**: 782. ill.

The original description is as follows: Mycelium forming compact tufts 1–1.5 mm in diameter, burnt sienna, becoming confluent and ef-

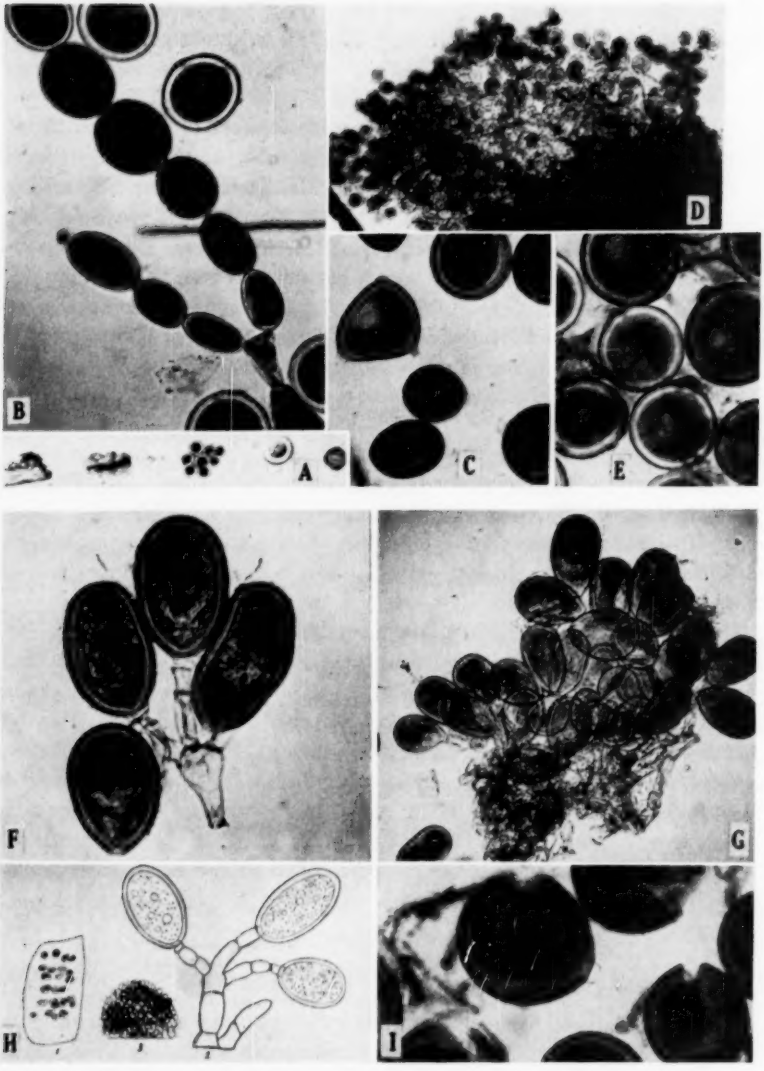


FIG. 2. A–D. *Sphaerosporium lignatile* Schw. A, original illustration reduced one-half from Trans. Am. Phil. Soc. **4**: 303; B, C, × 250, from collection of West and Murrill 1940, N. Y. Botanical Garden Herb., B mounted in 7% KOH and stained with phloxine, C mounted in lacto-phenol stained with cotton blue; D, × 58, TYPE from Academy of Natural Sciences, Philadelphia; E, × 250, *Oidium megalosporum* B. & C. AUTHENTIC, coll. Dr. Billings, ex Herb. Curtis; F, G, H,

fused; hyphae creeping, subhyaline to yellowish, short, septate, branched, densely intertwined, 5–7 μ thick; conidia subglobose to obovoid, smooth, thick walled (1.5–2 μ), orange, 14–30 × 11–20 μ, with coarsely granular contents.

On wood and bark. China.

This organism must be placed in the synonymy of *Allescheriella crocea* (Mont.) Hughes (**1951**). Compared microscopically and macroscopically, two collections from Hughes, determined by him as *A. crocea*, IMI *38104(a)* and IMI *38144* (FIG. 1, L), are similar to two collections from China, determined by Teng as *C. sinensis*, Teng. *2538* (FIG. 1, K) and Deng *5604* (FIG. 1, J). They all show glistening, orange-brown, thick-walled spores borne in clusters, in color in mass near "Mars Orange" or "Mahogany Red" (Ridgway). Spores of the *C. sinensis* collections measured 20.8–26 × 15.6–20.8 μ, and 18.2–20.8 × 11.7–15.6 μ; those of *A. crocea* measured 16.9–23.4 × 13 μ, and 15.6–23.4 × 11.7–15.6 μ, with walls 1.4–2.1 μ thick.

Doubtful species:

PROTOMYCES (EXOMYCES) PITHYOPHILUS Karst. 1884. Hedwigia **23**: 40.

Shortly after Karsten described this organism, and in the same volume (**1884**, p. 89), he stated that it might belong to the genus *Sphaerosporium* Schweinitz. Saccardo (**1892**, p. 512) suggested its disposition in *Coccospora* Wallroth. Actually no record appears of any new combinations ever having been made. Since the type of *Protomyces* (*Exomyces*) *pithyophilus* is no longer available, it is impossible to properly dispose of this name.

CONSIDERATION OF THE SYNONYMY OF COCCOSPORA WALLR.

1. SPHAEROSPORIUM Schweinitz. 1832.

Saccardo (**1886**) noted the strong resemblance of *Sphaerosporium* to *Coccospora*, although he did not consider the two synonymous. Then von Höhnel (**1911**) reduced *Sphaerosporium* to the synonymy of *Cocco-*

Bactridiopsis ulei P. Henn.: F, × 250, G, × 125, TYPE, H, original illustration reduced one-half from Hedwigia **43**: 397, showing habit sketch, sporodochium, and conidiophore; I, × 250, *Coccospora ulei* (P. Henn.) v. Höhnel, I.M.I. *38172(b)* collected by S. J. Hughes, mounted in lacto-phenol stained with cotton blue. D, E, F, and G mounted in Hoyer's solution and stained with acid fuchsin.

spora which he believed incorrectly to antedate it. This course was adopted by Clements and Shear (**1931**); but Shear (**1939**) pointed out the error and, believing *Coccospora* and *Sphaerosporium* to be distinct genera, re-established the genus of Schweinitz. Shear based his opinion on an examination of an isotype of *S. lignatile* Schw. and on his interpretation of Wallroth's description. Our re-examination of the type specimens (FIG. 2, D) confirms Shear's opinion.

Berkeley (**1869**) independently described *Oidium megalosporum* from a collection of an organism identical with *Sphaerosporium lignatile*. In so doing, this author noted the catenate character of the spores, which had been overlooked by Schweinitz. Linder (**1942**), after studying specimens of *O. megalosporum* from the Curtis collection, placed it in the synonymy of Schweinitz's earlier name. An examination of isotype (FIG. 2, E) and authentic material from the Curtis collection by the present authors confirms Linder's opinion.

Since *Sphaerosporium lignatile* has been the organism upon which the concept of *Coccospora aurantiaca* of many authors has been based, and because Schweinitz failed to recognize the catenate nature of the aleuriospores, an emended description of *S. lignatile* is presented here.

SPHAEROSPORIUM LIGNATILE Schweinitz. 1832. Trans. Am. Phil. Soc. n.s. **4**: 303. ill.

Myxosporium aurantiacum Rabenhorst. 1844. Krypt. Fl. I. Aufl. **1**: 41.

Oidium megalosporum Berkeley and Curtis. 1869. Jour. Linn. Soc. Lond. **10**: 363.

Protomyces xylogenus Saccardo. 1877. Mich. **1**: 14.

Sterile hyphae mostly within the substratum, external only as a basal cushion below the aleuriospores, hyaline, septate, branching, somewhat torulose in appearance; sporodochia or sporodochioid clumps 0.5–2 mm in diameter, hemispherical, usually separate, sometimes confluent, "Orange-Cinnamon" to "Ochraceous-Buff" in color (Ridgway); aleuriospores formed by the maturation of torulose elements of the fertile hyphae, catenate, older spores globose (36–50 μ in diam.), younger spores often ovate-truncate (38–66 $\times$ 33–45 μ), all spores having small flattened scars indicating points of attachment to other spores; spores smooth, thick-walled, wall strongly refractile in microscope preparations, central protoplasm faintly or distinctly granular; spores distinctly pale golden-yellow by transmitted light, easily broken by pressure upon coverglass.

Collections of this fungus made by the senior author and by Dr. G. W. Martin of Iowa differ from those of Schweinitz only in that fresh speci-

mens have sporodochia which are yellowish in appearance rather than cinnamon-ochraceous as in the type, and are somewhat gelatinous when fresh. However, this lighter "Ochraceous-Buff" color is not confined to fresh collections. It is found in six specimens in the Herbarium of the New York Botanical Garden, dating from 1889, 1891, 1893, 1932, and 1940. We do not regard this as a difference worthy of specific recognition since the spores of the type and of recent collections are microscopically identical in size, color, and morphology.

The important feature of the present emendation is a recognition of the catenate character of the spores. All spores have one, two, or three small, flattened, faintly protruding scars indicating their points of attachment to other spores. This is seen both in the type and in recent specimens (FIG. 2, C). In one of the specimens (N.Y.B.G. coll. West and Murrill, 1940) the manner of spore formation was observed (FIG. 2, B). It appears to be as described above. Repeated attempts to culture this fungus in order to observe this point more thoroughly have been unsuccessful.

2. PROTOMYCES Unger. 1833. Exanth. Pfl. p. 341.
Protomyces xylogenus. Sacc. 1877. Mich. **1**: 14.

Protomyces is a genus of parasitic fungi (Taphrinales) forming large, thick-walled, overwintering chlamydospores in the tissues of the host (Mix, 1949). As such, there is no room for organisms whose habitat is rotting wood.

P. xylogenus had been placed in the synonymy of *Coccospora aurantiaca* Wallr. by Saccardo (**1886**), and this arrangement was followed by Lindau (**1907**). However, Shear (**1939**) thought it to be *Sphaerosporium lignatile* Schw. It is now referred to this synonymy, since Saccardo's description and illustration clearly indicate a fungus like the one described by Schweinitz.

3. ALLESCHERIELLA Hennings. 1897. Hedwigia **36**: 244.
A. crocea (Mont.) Hughes. 1951. Mycol. Pap. No. 41, CMI, 17 pp.

Allescheriella has been re-established in a recent discussion by Hughes (**1951**), and has been shown to bear no resemblance whatsoever to *Coccospora aurantiaca* Wallr. *A. crocea* (Mont.) Hughes had previously been discussed under the name *Oidium simile* Berk. by Linder (**1942**). Hughes presented evidence that Linder had wrongly based his concept of *O. simile*, and concluded that the species should be removed

from *Oidium*. In any event, *Allescheriella* can no longer be considered for organisms in the *Coccospora* group.

Hughes described *A. crocea* as having conidia globose, subglobose, obovoid, or flask-shaped with markedly truncate base, 14–27 × 11–19 μ, having thick brownish walls when mature; spores borne at the tip of the conidiophore or its lateral branches as "blown-out ends"; conidiophores hyaline, simple or branched, at first yellow, the young pustules finally rust-colored. There is no indication that the conidia are borne in chains.

4. BACTRIDIOPSIS Hennings. 1904. Hedwigia **43**: 397.
B. ulei Henn. 1904. Hedwigia **43**: 397.

As Shear (**1939**) has pointed out, von Höhnel (**1911**), by a comparison of descriptions and illustrations, concluded that *B. ulei* Henn. should be placed under the synonymy of *Coccospora ulei* (P. Hennings) v. Höhnel, and was possibly identical with *Sphaerosporium lignatile* Schw. However, no evaluation of authentic material had been made. There is some similarity in the thick-walled spores of *S. lignatile* and *B. ulei* but they cannot be considered identical here. In the present study, the type specimen of *B. ulei* Henn., collected by Ernest Ule on rotten wood in Brazil in November 1900, has been examined. The spores are large, single-celled, ovate to ellipsoid, truncate at the base, hyaline, containing a granular cytoplasm, measuring 57–128 × 40–80 μ; spore walls smooth, 3.9–6.5 μ thick; spores borne singly at the tips of short, simple or slightly branched, hyaline, thin-walled conidiophores which arise as a sporodochium. Conidiophores usually show two septations just below the base of the spores, one at the point of abstriction of the spore and the other approximately 5–9 μ below, with this septate section often remaining attached to the spore when it becomes separated from the conidiophore. The growth markedly resembles that of *Sphaerosporium lignatile* Schw., except that the spores are larger, definitely elliptic and are not produced in chains (FIG. 2, F, G, H, I). In his original description Hennings pointed to the similarity between this species and *Bactridium flavum* Kunze but keyed them separately because of the larger non-septate spores of *Bactridiopsis ulei*. This fungus resembles species of *Bactridium* except that it lacks cross walls.

MATERIAL EXAMINED

Allescheriella crocea (Mont.) Hughes

1. On *Hevea brasiliensis* (Euphorbiaceae), Tarkwa, Gold Coast Colony, 12 May 1949, coll. and det. S. J. Hughes (*558*), Herb. I.M.I. *38104(a)*; through the

courtesy of Mr. E. W. Mason, Commonwealth Mycological Institute, Kew, England.

2. On rotten wood, Tafo, Gold Coast Colony, 14 June 1949, coll. and det. S. J. Hughes (*1242*), Herb. I.M.I. *38144;* through the courtesy of Mr. E. W. Mason, Commonwealth Mycological Institute, Kew, England.

Bactridiopsis ulei Henn., on rotten wood, Brazil, 1900. TYPE. E. Ule *2838*, Herbarium Brasiliense (Staatsinstitut für Allgemeine Botanik, Hamburg, Germany); through the courtesy of Prof. Dr. Walter Mevius.

Coccospora aurantiaca Wallr., on rotten wood, ?Germany, ?1833. TYPE. The Botanical Institute of Strasbourg; through the courtesy of Dr. R. E. Maresquelle.

C. casei Karst., on cheese, Finland. TYPE.

C. muscorum Karst., on moss, Finland, 1869. TYPE.

C. rosca Karst., on *Tricholoma saponaceum*, Finland, 1892. TYPE. The Botanical Museum of the University of Helsingfors; through the courtesy of Prof. Hans Buch.

C. rubiginosa Speg., on fallen trunk of *Passiflora coerulea*, Argentina, 1905. TYPE, *9210*, Universidad Nacional de La Plata Museo-Instituto Spegazzini, Colecciones Micologicas; through the courtesy of Prof. Lindquist.

C. sinensis Teng

1. On a stump, Ling-ku-sze Woods, Nanking, 28 July 1931, coll. and det. S. C. Teng *2538*. Reliquiae Herbarii Fungorum Sinensis *6091*. Undestroyed portion of the Chinese National Fungus Herbarium given to the Bureau of Plant Industry and Cornell University, 1939–40; through the courtesy of Dr. Richard P. Korf, Curator of the Herbarium, Cornell University.
2. On rotten wood, Tan-hsien, Hainan, 24 Oct. 1934, coll. S. Q. Deng *5604*, det. S. C. Teng, U.S.D.A. Bureau of Plant Industry, Pathological and Mycological Collections; through the courtesy of Mr. John A. Stevenson.

Oidium megalosporum B. & C.

1. On rotten polypore, Alabama, Peters (*1022*), 1856. ISOTYPE, ex Herb. Curtis (B. & C. packet *6094*), Farlow Herbarium, Harvard University; through the courtesy of the late Dr. W. L. White.
2. On rotten logs, Cuba, 1857. Coll. Wright, ex Herb. Curtis, Farlow Herbarium, Harvard University, AUTHENTIC; through the courtesy of the late Dr. W. L. White.
3. On rotten wood, Washington, D. C., Dr. Billings, ex Herb. Curtis, Brown University, AUTHENTIC; through the courtesy of Dr. Walter B. Snell.

Sclerotium applanatum Schw., on oak leaf, TYPE. The Academy of Natural Sciences of Philadelphia; through the courtesy of the late Dr. F. W. Pennell.

Sphaerosporium lignatile Schw., on rotten wood, Bethlehem, Pa., TYPE. The Academy of Natural Sciences of Philadelphia; through the courtesy of the late Dr. F. W. Pennell.

Six collections on dead wood, New York Botanical Garden; through the courtesy of Dr. D. P. Rogers, as follows:

1. West Haven, Conn., Oct. 1889, coll. and det. R. Thaxter.
2. West Haven, Conn., 24 Sept. 1891.
3. Grasmere, Fla., Mar. 1893.
4. Roaring Brook, Conn., 28 July 1893.

5. Highland Co., Ohio, 31 July 1932, W. B. Cooke coll. *882*, det. D. H. Linder (as *Coccospora aurantiaca* Wallr.).
6. Gainesville, Fla., 22 Jan. 1940, coll. West and Murrill, det. W. W. Diehl (as *Coccospora aurantiaca* Wallr.).

The authors are indebted to the individuals noted above, whose courtesy made it possible to study these valuable specimens.

The junior author is deeply grateful to Dr. Donald P. Rogers and to Dr. Emory G. Simmons for their consideration of this manuscript and for their very kind suggestions for its improvement.

PIONEERING RESEARCH LABORATORIES,
U. S. ARMY QUARTERMASTER CORPS,
PHILADELPHIA, PENNSYLVANIA

LITERATURE CITED

Berkeley, M. J. 1869. On a collection of fungi from Cuba. Part II, including those belonging to the families gasteromycetes, coniomycetes, hyphomycetes, physomycetes and ascomycetes. Jour. Linn. Soc. Lond. Bot. **10**: 341–392.

Bommer, E. and M. Rousseau. 1890. Contributions à la Flore Mycologique de Belgique. Bull. Soc. Roy. Bot. Belg. **29**: 205–302.

Clements, F. E. and C. L. Shear. 1931. The genera of fungi. 496 pp. New York.

Downing, M. H. 1953. *Botryotrichum* and *Coccospora*. Mycologia **45**: 934–940.

Gilman, J. C. 1945. A manual of the soil fungi. 392 pp. The Iowa State College Press.

Goddard, H. N. 1913. Can fungi living in agricultural soil assimilate free nitrogen? Bot. Gaz. **56**: 249–305. ill.

Hennings, P. 1897. Beiträge zur Pilzflora Südamerikas II. Hedwigia **36**: 190–246.

——. 1904. Fungi amazonici III. a cl. Ernesto Ule collecti. Hedwigia **43**: 351–399.

Höhnel, F. von. 1911. Fragmente zur Mykologie (XIII Mitteilung Nr. 642–718). Sitzungsber. Akad. Wien. I Abt. **120**: 402–403.

Hughes, S. J. 1951. Studies on micro-fungi. VII. *Allescheriella crocea, Oidium simile,* and *Pellicularia pruinata*. Mycol. Pap. No. 41, The Commonwealth Mycological Institute, 17 pp.

Karsten, P. A. 1884. Fragmenta mycologica XII. Hedwigia **23**: 39–40.

——. 1884. Fragmenta mycologica XIX. Hedwigia **23**: 88–89.

——. 1892. Fragmenta mycologica XXXVIII. Hedwigia **31**: 292–296.

——, **C. Roumeguere and P. Hariot.** 1890. Fungilli novi. Rev. Mycol. **12**: 79–80.

Lindau, G. 1907. In Rabenhorst's Kryptogamen-Flora. Vol. 8, 852 pp. Leipzig.

Linder, D. H. 1942. A contribution towards a monograph of the genus *Oidium* (Fungi Imperfecti). Lloydia **5**: 165–207. ill.

Mix, A. J. 1949. A monograph of the genus *Taphrina*. U. of Kansas Sci. Bull. 33, pt. 1, No. 1, 167 pp.

Montagne, C. 1845. In M. Pamon de la Sagra. Hist. Phys., Polit., et Nat. l'Ile de Cuba. Botanique—Plantes Cellulaires. p. 307.

Oudemans, C. A. J. A. 1919. Enumeratio Systematica Fungorum. Vol. 1, 1230 pp. The Hague.

Raper, K. B. and C. Thom. 1949. A manual of the Penicillia. 875 pp. Baltimore.

Saccardo, P. A. 1877. Fungi Veneti Novi vel Critici vel Mycologiae Venetae Addendi, Series VI. Michelia **1**: 1–73.

——. 1886. Syll. Fung. Vol. 4, 807 pp. Padova.

——. 1888. Syll. Fung. Vol. 7, 882 pp. Padova.

——. 1892. Syll. Fung. Vol. 10, 964 pp. Padova.

Schweinitz, L. D. von. 1832. Synopsis fungorum in America boreali media degentium, secundum observationes Ludovici Davidis de Schweinitz. Trans. Am. Phil. Soc. n.s. **4**: 141–316.

Shear, C. L. 1939. Mycological notes III. Mycologia **31**: 327–329.

Spegazzini, C. L. 1910. Mycetes Argentinenses. Series V, in Anal. Mus. Nac. Buenos Aires, series 3, **13**: 329–467.

Teng, S. C. 1936. Sinensia (Nanking) **7**: 782.

Unger, F. J. A. N. 1833. Die Exantheme der Pflanzen, 422 pp. Vienna.

Wallroth, F. G. 1833. Flora Crypt. Germ. **2**: 176. Nuremberg.

White, W. L. and M. H. Downing. 1951. *Coccospora agricola* Goddard, its specific status, relationships, and cellulolytic activity. Mycologia **43**: 645–657.

MORPHOLOGY OF SPORE FORMS AND HETEROECISM IN PUCCINIA CACAO

M. J. Thirumalachar and M. J. Narasimhan

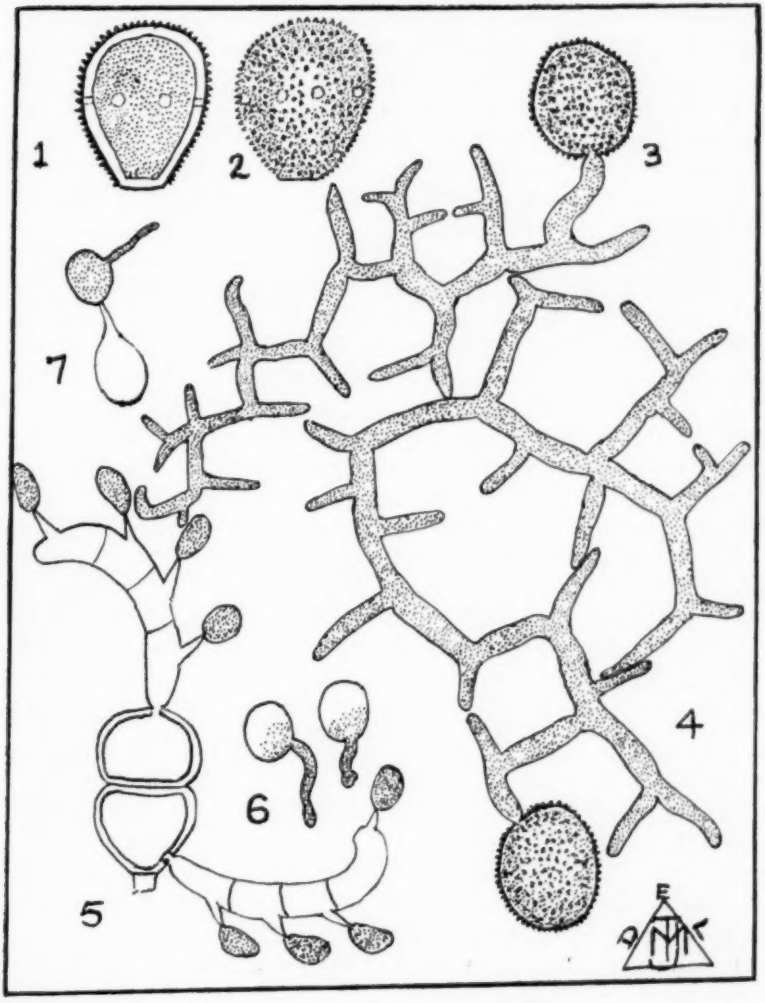
(with 8 figures)

Puccinia cacao McAlp. causes the leaf rust of *Hemarthria compressa* (Roxb.) Kunth. (*Rottboellia compressa* Roxb.), a common grass growing in marshy places. This rust has been reported from several places in India (Butler & Bisby **1931**) and is also known to occur in Australia (McAlpine **1906**), China (Tai **1947**) and Japan (Hiratsuka **1952**). Interest in this rust was aroused in connection with finding of the alternate host for the aecial stage of a systemic rust on *Hygrophila spinosa* T. And. (*Asteracantha longifolia* Nees.), a thorny plant inhabiting the marshy regions in the rice fields. This aecial stage on *H. spinosa* is identical with *Aecidium manilense* Arth. & Cumm. (Arthur & Cummins **1936b**) reported hitherto only from the Philippines. Field observations and cross inoculation studies revealed that *Puccinia cacao* developed pycnial and aecial stages on the acanthaceous host *Hygrophila spinosa* and uredial and telial stages on *Hemarthria compressa*. A brief account of these studies is presented here.

Life-cycle on Hemarthria compressa

The uredial stage begins to appear on the leaves in December–January as minute pulvinate spots on the upper surface. Due to rupture of overlying epidermis the sori become naked, releasing a brown spore mass. The infection becomes severe in the field by the end of January as a result of secondary infection of the host by the urediospores. The teliospores are developed during the month of February, first associated within uredia and later formed in separate sori. The telia are erumpent and pulverulent, slightly darker in color than the uredia. Owing to the fragile nature of the pedicels, the teliospores become separated from the spore bed and are dispersed in wind currents like urediospores.

Uredia and telia are mostly epiphyllous, subepidermal, erumpent, pulverulent, and paraphysate. The urediospores are ovate-ellipsoid, chestnut-brown in color, with epispore covered with close echinulations and



FIGS. 1, 2. Urediospores, × 750. 3, 4. Germinating urediospores, × 500. 5. Germinating teliospore, × 750. 6. Sporidia germinating by germ tubes, × 1000. 7. Development of secondary sporidia, × 1250.

The magnifications given above should be reduced by about 10% for Figs. 1–6, and about 5% for Fig. 7.

possessing 3 to 4 equatorial germ pores (FIGS. 1, 2). The teliospores are reddish-brown, 2-celled, slightly or not constricted at the septa, rounded at the apex, not apically thickened but with a wall uniformly thin. The pedicels are hyaline, less than 10 μ long, fragile, and deciduous. The germ pore is apical in the upper cell and located at the side of the pedicel in the lower cell. This feature becomes apparent only at the time of teliospore germination (FIG. 5). Such a disposition of the germ pore in the lower cell has been described by Arthur & Cummins (**1936a**) in *Puccinia subcentripora*.

The urediospores germinate readily when placed on droplets of water on slides and incubated at room temperature (20° C). The stout germ tube emerges from the germ pore and grows rapidly. The branching pattern of the germ tubes conforms to an unusual type, the ultimate branchlets being disposed of in a manner similar to the net-like structure of the green alga *Hydrodictyon*. There are no anastomoses of the germ tubes or the branches to form a true net, but the ends of the branchlets slide across each other, closely adpressed, to give the shape of a net (FIGS. 4, 8B). The germ tube at first shows zig-zag branchings and remains unseptate. The secondary branches arise from angles of the bends of these main axes (FIG. 3). These secondary branches in turn show bends in a manner to form hexagons or pentagons. There are definite indications that the branchlet at one point stimulates formation of a similar branchlet on the oppositely side of the germ tubes. This phenomenon takes place among branchlets of germ tubes of either the same spore or different spores, so that the hexagonal or pentagonal pattern is brought about. These long germ tubes remain mostly unseptate.

The teliospores germinate readily within 12 hours when placed in moist chambers at room temperature. The two cells of the spore germinate simultaneously bearing sporidia on sterigmata (FIG. 5). The sporidia in most cases germinate in situ, developing infection hyphae, or produce secondary sporidia (FIGS. 6, 7).

Rusted leaves bearing uredia and telia were collected and placed in the field to study oversummering of the rust. The urediospores lose their viability within a week during the month of May when the day temperature goes up to 107° F. Study of the teliospore material indicated that after the commencement of the rainy reason in July, most of the teliospores germinated due to their non-dormant nature. Between the months of June and November, no rusted leaves of *Hemarthria compressa* were found in the field on the growing plants.

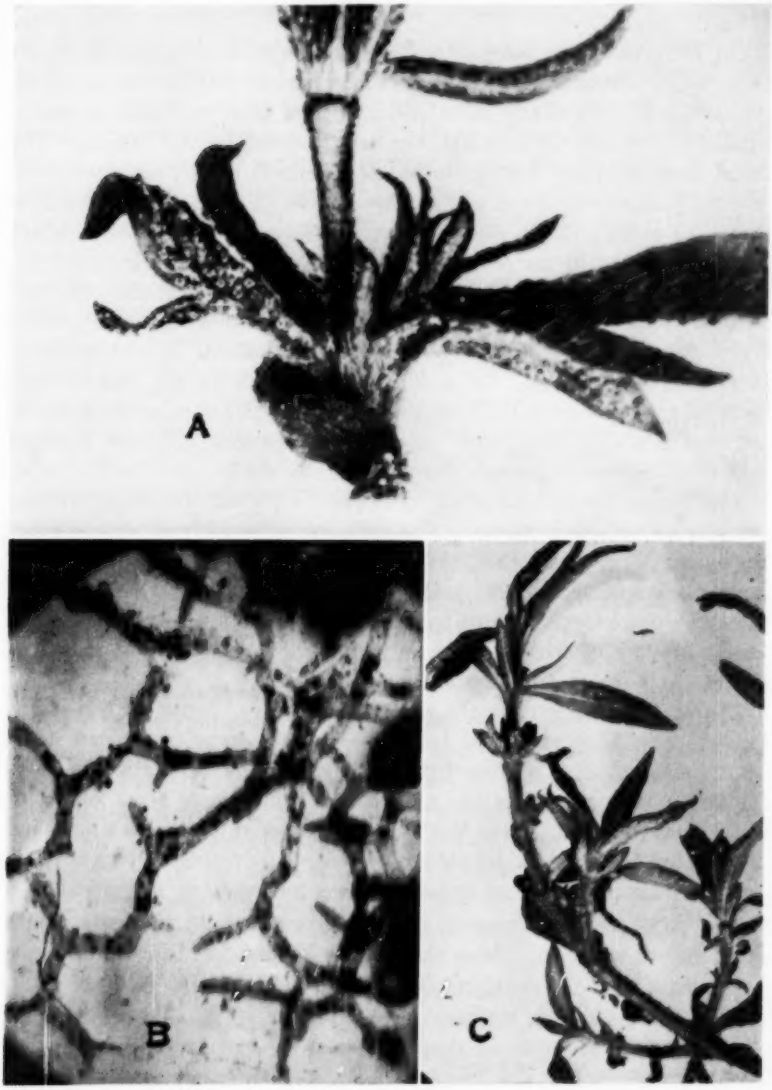


FIG. 8. A. Artificially inoculated shoot of *Hygrophila spinosa* after 25 days showing aecia and beginning of systemic infection, × 2. B. Photomicrograph of germinating urediospores, × 500. C. Systemically infected shoot of *H. spinosa* showing suppression of spines, × ½.

LIFE-CYCLE ON HYGROPHILA SPINOSA

On *H. spinosa* the fungus produces a systemic infection of shoots (FIG. 8C). From the point of initial infection on the leaf, the mycelium grows rapidly and affects the apical meristem, thus becoming systemic. In due course the entire plant becomes invaded by the fungus. The aerial shoot portions dry up during the summer month of May. Following the commencement of the rainy season in July, new shoots arise from root stocks. Such shoots arising from root stocks of plants which showed infection in the previous season, reveal systemic infection.

Infected shoots are slightly paler in color than the healthy ones and show interesting morphological variations. The whorls of spines which are modified bracts and bracteoles are very prominent in healthy plants of *Hygrophila spinosa*. In systemically infected plants, the development of spines is completely suppressed (FIG. 8C) and such plants do not bear flowers. The rusted plants have been found to have a longer period of vegetative growth than the healthy ones.

The pycnia are subepidermal, erumpent, with well-developed ostiolar paraphyses and pycnospores. The aecia are subepidermal, cupulate and peridiate. The aeciospores are developed in chains and at maturity large masses of aeciospores are ejected. The seasonal development of these two spore forms appears to be influenced by temperature conditions. In the absence of critical study on temperature relationships, only field observations are reported here.

Pycnia are first seen in the field during July–August on leaves and tender stems of systemically infected plants growing from root stocks bearing infection. They are hypophyllous and chiefly distributed along the veins, appearing as yellow globules. As the plants grow larger, up to two feet in height, all the leaves become covered with pycnia and none of them are accompanied by aecia. Extensive field observations between July and the end of October have shown hundreds of systemically infected plants, the entire plant being covered with pycnia without the development of aecia. These plants are easily mistaken for healthy ones in the field, since the conspicuous aecia are lacking. The infected plants can be easily detected, however, by the lack of spines and slight paling of the leaves. Microscopic examination of the material showed that no pycnospores or well developed ostioles were present, and the sterile sporophores filled up the pycnial cavity. Arthur and Cummins (**1936b**), in their study of material collected in Philippines, state that no pycnospores were seen and that in some of the leaves only pycnial stages were seen covering the entire leaf surface without being accompanied by aecia.

These observations indicate a similar type of development to that noted in the present study. It is interesting that a systemically infected plant growing to a height of 2 to 3 feet should bear only pycnial stages for three months of the growth period up to the end of October, and immediately after the advent of cold weather in November should produce pycnia accompanied by aecia. During the month of August, some of the systemically infected plants show little or no pycnial development, so that the progress of infection in the developing leaves cannot be detected. However, microscopic examination of such leaves near the mid-rib region reveals the hyphae of the rust.

As already stated, normal pycnia, as contrasted with the abortive pycnia, are formed in November following the commencement of the cold season (day temperature up to 80° F and night temperature going down to 60° F). These pycnia are orange-yellow and when examined with a hand lens, show tiny globules of nectar secreted at the mouth. The aecia follow the pycnia in development, erupting masses of aeciospores. The aeciospores germinate readily in water and produce long germ tubes.

INOCULATION EXPERIMENTS

The connection between the stages of the rust on *Hemarthria compressa* and *Hygrophila spinosa* was first suspected on the basis of field studies and later established from cross-inoculation experiments. Sporidial inoculations were carried out on young seedling plants of *Hygrophila spinosa* grown in pots. Sporidial suspensions from germinating teliospores were placed on young leaves and the inoculated plants were incubated in moist chambers for 24 hours. Observations after 15 days indicated slight paling of the leaves and the development of a few pycnia appearing as tiny orange-yellow globules. In due course the hyphae moved down the petiole to the stem and incited the systemic infection of the apical meristem. Since the inoculations were carried out during February, the pycnia were accompanied by abundant aecial development (FIG. 8A). This behavior of the mycelium moving down to the stem from the leaf and inciting systemic infection is reported in other rusts, such as *Puccinia minussensis* and *P. butleri*.

Young plants of *Hemarthria compressa* were inoculated with aeciospores from *Hygrophila spinosa*. The plants to be inoculated were first sprayed with water and dusted with aeciospores. The inoculated plants were incubated in moist chambers for 24 hours. Observation after 15 days showed development of uredial pustules on the upper leaf surface, while none were present in plants kept as controls.

CONCLUSIONS AND SUMMARY

The present study has shown that *Puccinia cacao* McAlpine is a heteroecious rust developing uredial and telial stages on *Hemarthria compressa* and pycnia and aecia on *Hygrophila spinosa*. The aecial stage of this rust has previously been reported only from the Philippines under the name *Aecidium manilense* Arth. & Cumm. while the uredial and telial stages have been reported on *Hemarthia compressa* from several places in the orient. Since the aecial stages on *Hygrophila* are easily overlooked in the field, due to the non-formation of aecia for long periods in spite of systemic infection, a search for spineless *Hygrophila* plants in places where *P. cacao* has been reported on the grass host may reveal the presence of aecial stages also. This is based on the fact that systemically infected plants of *Hygrophila* have no spines.

Field studies have indicated that the rust is perennial on the aecial host and, at least under conditions prevailing in India, oversummers as dormant systemic mycelium within the root stocks of *H. spinosa*. The heteroecious nature of the rust has been confirmed by cross-inoculation experiments.

LITERATURE CITED

Arthur, J. C. and G. B. Cummins. 1936a. Philippine rusts in the Clemens collection 1923–1926. I. Philippine Jour. Sci. **59**: 437–448.

—— ——. 1936b. Philippine rusts in the Clemens collection 1923–1926. II Philippine Jour. Sci. **61**: 463–488.

Butler, E. J. and G. R. Bisby. 1931. The fungi of India. Sci. Monogr. 1, Imperial Council of Agricultural Research, India, p. 65.

Hiratsuka, N. 1952. Uredinales of Kiushu. Contributions to the rust flora of eastern Asia—IV. Mem. Faculty Agric. Tokyo Univ. Education **1**: 1–95.

McAlpine, D. 1906. The rusts of Australia, their structure, nature and classification, p. 295.

Tai, F. L. 1947. Uredinales of Western China. Farlowia **3**: 95–139.

POLYPORUS MONTAGNEI AND CYCLOMYCES GREENEI

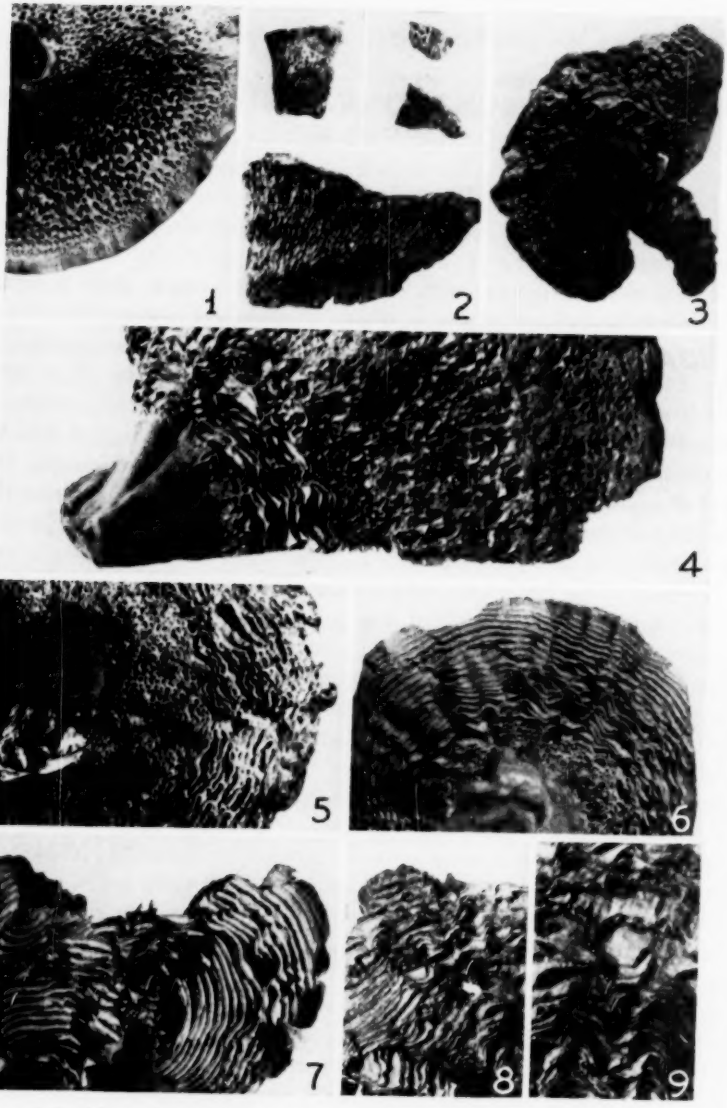
ROBERT L. GILBERTSON

(WITH 9 FIGURES)

The stipitate polypores with distinctly brown context form a small, quite sharply defined group in which all members are poroid except *Cyclomyces greenei* Berk., which is sometimes completely concentrically lamellate. This species was described from Massachusetts (**3**, p. 306) and is known from the United States and Japan (**8**, p. 633). Berkeley assigned the fungus to the genus *Cyclomyces* Kunze, although it differed markedly in its terrestrial habitat, stipitate habit, colored spores, and lack of setae from the one other species known at that time from the tropics. These differences led Murrill (**12**, p. 423) to erect a new monotypic genus, *Cycloporus*.

Frequently the hymenial configuration of this fungus is poroid in part, a condition which suggests that it may be related to a poroid member of the group. This variable hymenial configuration has long been known. The type shows some evidence of the poroid condition (FIGS. 8, 9), and other mycologists have regularly reported variations from the lamellate condition. Patouillard (**15**, p. 50) believed that its affinities were near the species of the *Perennes* section of the genus *Polystictus* Fries, and that the lamellate configuration was of little value as a generic character. Murrill (**13**, p. 370) described the tubes as being "at first polygonal, soon becoming continuous concentric furrows." Ames (**1**, p. 245) believed the lamellae resulted from aborted radial pore walls, and also noted its similarity to species of *Polystictus*, "especially those with a thick pileus as *P. obesus*." A specimen with a "polyporoid hymenium" is reported by Lloyd (**8**, p. 1260). Overholts (**14**, p. 117) describes the tubes as "soon breaking up to form . . . concentric . . . lamellae."

Originally described from France (**10**, p. 341), *Polyporus montagnei* Fries is reported by Pilát (**16**, p. 582) from several additional European countries, and by Overholts (**14**, p. 117) from the eastern United States. Studies of *P. montagnei* have been made by Bambeke (**2**) and Coker (**6**). The fungus shows a rather distinct morphology, and only minor variations in hymenial configuration are described by various authors. Quelét (**17**, p. 252) states "pores irréguliers, en forme d'alvéoles peu



FIGS. 1–9. Hymenial configuration of *Polyporus montagnei* and *Cyclomyces greenei*. All × 1 except FIG. 9. 1. Strictly poroid, typical of *P. montagnei*. W. C. Coker *12734* (NCU). 2. Fragments of type of *P. montagnei* (PC). 3. Irregular distribution of lamellate and poroid areas. H. R. Totten *5659* (NCU). 4. Lamellate near stipe. E. B. Sterling *55428* (US). 5. Poroid near stipe and at margin.

profonds." Lowe (**9**, p. 43) describes the pores as being "at first circular, soon angular to daedaloid." According to Coker (**6**, p. 100) the tube mouths are "of unequal size . . . often elongated near the stem." Bresadola (**4**, p. 240; **5**, p. 961) believed the type of *P. montagnei* to be the same as *P. perennis* Fries. The type collection of *P. montagnei* (FIG. 2), however, agrees with the current American concept of the species and differs markedly from *Polyporus perennis*, particularly in the spores and context hyphae. Ellis and Everhart (**7**, p. 125) described a thick form of this plant as *Polystictus obesus*, and Murrill (**11**, p. 347) described a form with a short, thick stipe as *Coltricia memmingeri*.

It was noted that the descriptions of *Polyporus montagnei* and *Cyclomyces greenei* were very similar except for variations in the hymenial configuration. Therefore a comparison was made of the morphological characters of typical representatives of these two species, as well as those with intermediate configuration. A total of 160 collections has been studied to determine the dependability of the concentric lamellae as a diagnostic character for *C. greenei*, and to learn if other characters have value in separating the two species.

In addition to specimens typical of lamellate *Cyclomyces greenei* (FIGS. 7, 8) and poroid *Polyporus montagnei* (FIGS. 1, 2), a series of intergradations between the two forms of pore surface was found. Some specimens were lamellate near the stipe and poroid at the margin (FIG. 4), some have the opposite arrangement (FIGS. 5, 6), and still others showed an irregular arrangement of poroid and lamellate areas (FIG. 3). Hymenial configuration therefore cannot be used as a diagnostic criterion for these two species.

No consistent difference in the other macroscopic features of the two species was observed. The size, consistency and thickness of the fruiting body, the color of the pileus, context and tubes, the nature of the surface of the pileus, and the appearance of the stipe showed the same range of variation in the specimens studied.

No dependable criteria for the separation of the two species on the basis of microscopic characters were found. In both species the hyphae of the context are pale brown in KOH, thin-walled, septate, and 6–14 μ in diameter. Spores are pale-brownish in KOH, smooth, ellipsoid, and 6–7.5 μ wide and 9–15 μ long.

G. F. Atkinson *1* (CU). 6. Poroid only near stipe. F. V. Coville and O. F. Cook *64* (NY). 7. Strictly lamellate, typical of *C. greenei*. H. Sherwin *13282* (NCU). 8. Part of type of *C. greenei* (K). 9. Portion of type of *C. greenei* enlarged to show pores, $\times 2$ (K).

On the basis of the intergradation from poroid to lamellate hymenophore, and the similarity of all other morphological features, it appears that *Polyporus montagnei* and *Cyclomyces greenei* represent the extremes in variation of a single species. The poroid condition, being the more common, appears to be typical and may be designated as *Polyporus montagnei* var. **montagnei.** A name for the lamellate condition seems desirable and it is proposed that it be called *Polyporus montagnei* var. **greenei** (Berk.) Gilbertson, comb. nov.

Collections of the two varieties were made available for study through the courtesy of the curators of the herbaria of the University of North Carolina, Chapel Hill, North Carolina (designated by the symbol NCU), the New York Botanical Garden, New York, New York (NY), Cornell University, Ithaca, New York (CU), the Bureau of Plant Industry and the Smithsonian Institution, Beltsville, Maryland (US), the Royal Botanic Gardens, Kew, England (K) and the Museum National d'Histoire Naturelle, Laboratoire de Cryptogamie, Paris, France (PC).

The author wishes to express his appreciation to Dr. J. L. Lowe, under whose direction this work was done.

STATE UNIVERSITY OF NEW YORK,
COLLEGE OF FORESTRY,
SYRACUSE, NEW YORK

LITERATURE CITED

1. **Ames, Adeline.** 1913. A consideration of structure in relation to genera of the Polyporaceae. Ann. Mycol. **11**: 211–253.
2. **Bambeke, Ch. v.** 1909. Sur *Polystictus cinnamomeus* (Jacq.) Sacc. et *Polystictus montagnei* Fries. Soc. Roy. Bot. Belg. Bull. **46**: 1–24.
3. **Berkeley, M. J.** 1845. Decades of fungi. Dec. VIII—X. Australian and North American fungi. London Jour. Bot. **4**: 298–315.
4. **Bresadola, J.** 1916. Synonymia et adnotanda mycologica. Ann. Mycol. **14**: 221–242.
5. ——. 1931. Iconographia mycologica, vol. 20.
6. **Coker, W. C.** 1946. The United States species of *Coltricia*. Elisha Mitchell Sci. Soc. Jour. **62**: 95–107.
7. **Ellis, J. B. and B. M. Everhart.** 1897. New species of fungi from various localities. Torrey Bot. Club Bull. **24**: 125–137.
8. **Lloyd, C. G.** 1910–1924. Mycological Writings **3–7** (Myc. Notes 32–75): 413–1364. 1908–1925.
9. **Lowe, J. L.** 1942. The Polyporaceae of New York State (except *Poria*). N. Y. State Col. Forestry, Syracuse Univ., Tech. Pub. 60.
10. **Montagne, C.** 1836. Notice sur les plantes cryptogames recémment découvertes en France, contenant aussi l'indication précise des localités de quelques espéces les plus rares de la Flore française. Ann. Sci. Nat. Bot. II. **5**: 337–348.

11. **Murrill, W. A.** 1904a. The Polyporaceae of North America—VII. The genera *Hexagona, Grifola, Romellia, Coltricia* and *Coltriciella.* Torrey Bot. Club Bull. **31**: 325–348.
12. ——. 1904. The Polyporaceae of North America—VIII. *Hapalopilus, Pycnoporus,* and new monotypic genera. Torrey Bot. Club Bull. **31**: 415–428.
13. ——. 1905. The Polyporaceae of North America—XI. A synopsis of the brown pileate species. Torrey Bot. Club Bull. **32**: 353–371.
14. **Overholts, L. O.** 1953. The Polyporaceae of the United States, Canada and Alaska. University of Michigan Press. (In press.)
15. **Patouillard, N.** 1896. Le genre *Cyclomyces.* Soc. Mycol. France Bull. **12**: 45–51.
16. **Pilát, A.** 1936–1942. Atlas des champignons de l'Europe III. Polyporaceae I.
17. **Quelét, L.** 1872–1875. Champignons du Jura et des Vosges.

PORIA COCOS, A WIDELY DISTRIBUTED WOOD-ROTTING FUNGUS

ROSS W. DAVIDSON AND W. A. CAMPBELL [1, 2, 3]

(WITH 1 FIGURE)

The sclerotia, or tuckahoes, of *Poria cocos* Wolf have been found in a number of localities in the South and East. They are usually associated with tree roots, and in a few instances have been collected in quantities during land clearing operations or during plowing of recently cleared land. For years the fungus was called *Pachyma cocos* Fr. since it was earlier known only in the sclerotial stage. Weber (**4**) in 1929 published a review of the literature dealing with the fungus and gave a list of the many names that had been applied to it as well as information on distribution, habitat, and host range. At the time of his study, practically all information regarding the fungus was based on the sclerotial stage. The *Poria* stage was known only on sclerotia, as described by Wolf in 1922 (**5**). In Florida the sclerotia had been found on the roots of magnolia, grapefruit, oak, sweet orange and Eucalyptus. Other reported substrata, as indicated by Weber, included sumac, corn, fir, and cedar. The geographic distribution reported was given as New York to Florida west to Texas, Arkansas, and Kansas.

In 1942 Davidson *et al.* (**2**) reported the occurrence of *Poria cocos* in association with a brown butt and root rot of oaks. The fungus was isolated 45 times from 4 species of oaks and was obtained 5 times from rot samples from the Allegheny region, 25 times from the Appalachian region, and 15 times from the Middle West. The fungus isolated from these samples was identified as *Poria cocos*, by comparing the cultural characteristics of these isolates with those of the fungus obtained from a sclerotium collected near Annapolis, Maryland.

Jackson (**3**) in 1945 showed that sclerotia of *Poria cocos* were attached to the large roots of shortleaf pine (*Pinus echinata* Mill.) affected

[1] Senior Pathologists, U. S. Division of Forest Pathology, in cooperation with Colorado Agricultural Experiment Station and Rocky Mountain Forest Experiment Station, Fort Collins, Colo., and School of Forestry, University of Georgia, Athens, Ga.

[2] The writers are indebted to Frances Lombard for assistance with cultures and to the many individuals who furnished decay samples and sporophores of the fungus.

[3] Colorado Agricultural Experiment Station. Scientific Series, Paper No. 421.

by littleleaf disease. These sclerotia varied from one-half to two inches in length and appeared as irregular lumps on the surface of the bark and thus differed markedly in shape and size from the large tuberous tuckahoes usually encountered. Later Campbell (1) showed that these tuckahoes could also be found on the roots of healthy shortleaf pine. All identifications of the tuckahoes from pine roots were made by comparing isolates with those from the culture collection maintained by the Division of Forest Pathology. In all cases where sclerotia were found attached to the roots of living pines there appeared to be no associated decay.

Observations on decay of pines killed by littleleaf disease showed that many were soon affected by a brown cubical butt rot caused by *Poria cocos*. This rot usually caused the trees to break near the ground within a few years after the trees had died from littleleaf. However, this type of rot was not found at stump height in recently killed trees or on trees cut in the advanced stage of littleleaf. The number of dead trees showing brown rot caused by *P. cocos* varied according to locality. In some areas many of the dead trees developed brown butt rot; in others only white rots or rots not associated with *P. cocos* were present.

In some littleleaf areas the abundance of dead trees having butt and trunk rot caused by *Poria cocos* led to an extensive search for the *Poria* stage. Only one collection of a *Poria* associated with a brown rot of the *P. cocos* type was made in the South. This was on a littleleaf plot in Colbert County, Alabama, in September 1946. A littleleaf-killed tree had broken about 6 feet above ground so that the trunk had not fallen to the ground but had remained attached, leaving a protected area of exposed rot. A *Poria* had developed on the exposed rot surface. Cultures made from basidiospores, sporophore tissue, and the rot had the same cultural characteristics as isolates of *P. cocos* obtained from sclerotia. The morphological characters of the species of *Poria* collected in Alabama agreed closely with those of a *Poria*, suspected of being *P. cocos*, collected by J. A. Stevenson [4] in 1937 from the floor of a summer home near Annapolis, Maryland. Cultures from the two sporophores were also similar.

The widespread distribution of pine rotted by *Poria cocos* in the South and its prevalence on oak and other species suggested that the *Poria* stage might have been collected by earlier workers and possibly could be found in herbaria under another name. Accordingly the two collections listed above, plus specimens from a western white pine (*Pinus monticola* D. Don.) log collected in northern Idaho in 1949 (FIG. 1) and one collected

[4] Div. of Mycology and Disease Survey, U.S.D.A.

by W. W. Wagener on *Pinus lambertiana* Dougl. in California in 1948 (FP *94401*), were submitted to J. L. Lowe for comparison with other *Porias*. Dr. Lowe stated that the fungus found on logs in the field is morphologically comparable to that which develops on sclerotia in the laboratory and is not referable to any other named species of *Poria*. Undoubtedly *Poria* collected occasionally on logs can correctly be called *P. cocos* but such fruiting is not common.

During routine decay isolation studies carried on by the Division of Forest Pathology, *Poria cocos* has been isolated from a variety of hosts from most of the important timber areas of the United States. New

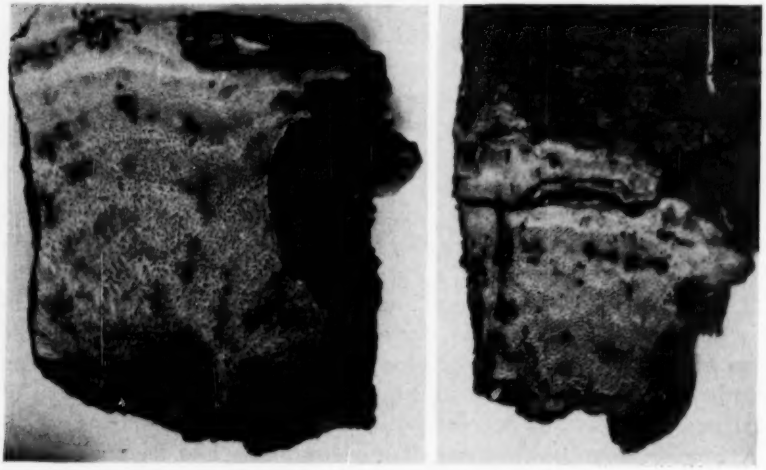


FIG. 1. Sporophore of *Poria cocos* from *Pinus monticola* log (FP *97438*).

hosts and locations of collections, in addition to those already given, are listed below.

Abies balsamea (L.) Mill., Deer River, Minn., L. L. Sluzalis, 1935.
Abies sp., Root rot, Black Mt. Expt. For., Calif., R. W Davidson, 1944.
Acer sp., Elk Co., Pa., W. A. Campbell, E. R. Roth and B. Sleeth, 1936.
Betula lutea Michx. L., Loretta, Wis., R. C. Lorenz, 1936.
B. papyrifera Marsh, Irving, Mass., W. A. Campbell, 1937.
Fagus grandifolia Ehrh., Allegheny Natl. Forest, Pa., 1936, and State College, Pa., W. A. Campbell, 1937.
Pinus monticola D. Don., Northern Idaho, Pole blight workers, 1948.
Tsuga canadensis L. Carr., Loretta, Wis., R. C. Lorenz, 1936.

T. heterophylla Sarg., Siskiyou Natl. Forest, Oreg., E. Wright, 1947.
Ulmus americana L., Rhinelander, Wis., R. C. Lorenz, 1936.

DIVISION OF FOREST PATHOLOGY, U. S. DEPARTMENT OF AGRICULTURE,
in cooperation with COLORADO AGRICULTURAL EXPT. STATION & ROCKY MT.
FOREST EXPT. STATION, FORT COLLINS, COLO., AND
SCHOOL OF FORESTRY, UNIVERSITY OF GEORGIA, ATHENS, GA.

LITERATURE CITED

1. **Campbell, W. A.** Fungi associated with the roots of littleleaf-diseased and healthy shortleaf pine. Phytopath. **41**: 439–446. 1951.
2. **Davidson, R. W., W. A. Campbell and D. B. Vaughn.** Fungi causing decay of living oaks in the eastern United States and their cultural identification. U. S. Dept. Agri. Tech. Bull. 785. 65 pp. 1942.
3. **Jackson, L. W. R.** Root defects and fungi associated with the little-leaf disease of southern pines. Phytopath. **35**: 91–105. 1945.
4. **Weber, G. F.** The occurrence of tuckahoes and *Poria cocos* in Florida. Mycologia **21**: 113–130. 1929.
5. **Wolf, F. A.** The fruiting stage of the tuckahoe, *Pachyma cocos*. Elisha Mitchell Sci. Soc. Jour. **38**: 127–137. 1922.

OBSERVATIONS ON TILLETIA CEREBRINA [1, 2]

W. N. SIANG [3]

(WITH 2 FIGURES)

Thirty-one species of *Tilletia* are currently recognized as occurring in North America (**2**). However, most of the species are necessarily included in this genus by apparent analogy on the basis of spore morphology and host symptoms, since characteristics of spore germination are known in only fourteen species. The more economically important species, e.g., *T. brevifaciens* G. W. Fisch., *T. caries* (DC.) Tul., and *T. foetida* (Wallr.) Liro, have been so studied. In addition, they have been successfully cultured on artificial media, and their cytology investigated (**6, 8**). In *T. elymi* Diet. and Holw., and *T. asperifolia* Ell. and Ev., different types of spore germination recently have been reported (**5, 7**). In the present paper, the characteristics of spore germination and nuclear cytology during spore germination of *T. cerebrina* Ell. and Ev. (*T. airae* Blytt) are described. This species parasitizes grasses in the genus *Deschampsia,* mainly in western United States, Alaska, and eastern Canada (Southampton Island) (**2**). It is also widely distributed on the European continent (**9**).

MATERIALS AND METHODS

The smut material used in this investigation was collected by George W. Fischer and associates. Two collections were used: one on *Deschampsia elongata* (Hook.) Munro ex Benth. (Myc. Coll. Bur. Plant Ind. no. *85556,*[4] McCall, Idaho, August 1, 1945); and another on *D. danthonoides* (Trin.) Munro ex Benth. (no. *85199,* Goldendale, Wash-

[1] This investigation was supported in part by funds provided for biological and medical research by the State of Washington Initiative Measure No. 171.

[2] Scientific Paper No. 1202, Washington Agricultural Experiment Stations, Pullman. Project No. 1038. From a portion of thesis submitted to The Graduate School of the State College of Washington in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[3] Formerly Research Assistant, Department of Plant Pathology, State College of Washington, Pullman.

[4] These numbers are the accession numbers of the Mycological Collections of the Bureau of Plant Industry, Soils and Agricultural Engineering, Beltsville, Md., where portions of each collection have been deposited.

ington, June 22, 1943). The smut balls were soaked in small vials of sterilized distilled water for 2 hours. Some of the well dispersed spore suspension was poured on 3 per cent Difco water agar in Petri dishes and incubated. The spores on the plates were examined at regular intervals during the course of their germination.

For cytological examination, the germinating spores were killed and fixed *in situ* when the desired stages were attained. Flemming's weaker

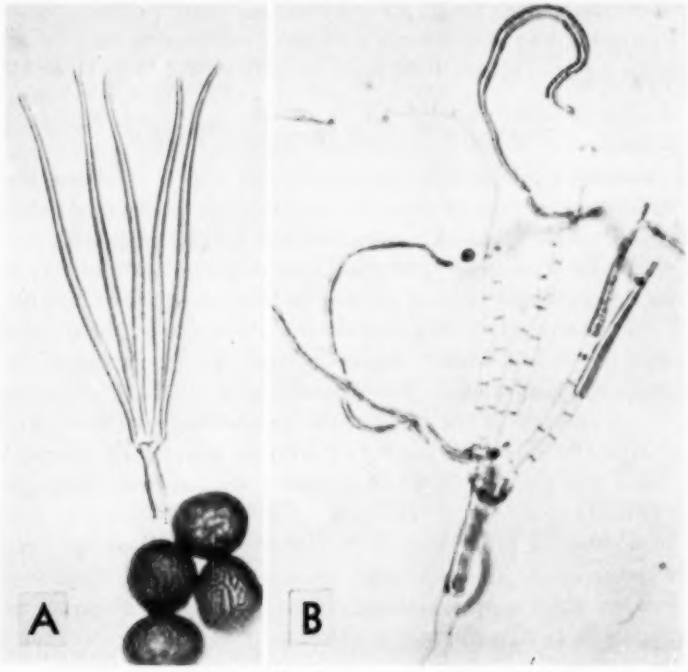


FIG. 1. Chlamydospore germination in *Tilletia cerebrina*. A. Showing primary sporidia. B. Primary sporidia evacuated and producing infection hyphae. × 1200.

solution gave better results than a number of other fixing fluids, particularly when followed by Heidenhain's hematoxylin stain. The excess fixing fluid was poured off after 20 minutes, which was sufficient to assure good fixation. The agar containing the fixed material was allowed to dry for a few minutes before being cut into small squares. These agar blacks were inverted and affixed to slides on which thin films of Haupt's adhesive were smeared. They were later removed by

dipping the slides in 10 percent hydrogen peroxide solution for 20 minutes, which simultaneously bleached the fixed spore material remaining on the slide.

Several nuclear stains with different methods of staining were tried. Heidenhain's iron hematoxylin gave the best results, especially when destaining was carefully done in acid alcohol. The stained material was then mounted in Piccolyte for microscopic examination.

For the isolation and handling of single primary sporidia, a Chamber's micromanipulator was used. Primary sporidia were isolated and paired in different combinations according to the modified procedure described by Holton (**4**). These isolated sporidia were incubated at 10 or 15° C.

CHARACTERISTICS OF SPORE GERMINATION

In an earlier report on spore germination of *Tilletia cerebrina*, Fischer and Hirschhorn (**3**) stated that germination could be obtained only after the spores had been soaked in water for eight weeks at 2–3° C. In the present test, however, presoaking was not essential. The spores began germinating on water agar at the end of four weeks when incubated at 5° C. The early stages of germination were similar to those described for other species of *Tilletia*. As in *T. elymi* (**5**), the characteristic feature of spore germination is the formation of a relatively small number of primary sporidia at the apex of the promycelium. These comparatively large (99.8–119.7 × 4.3–5.7 μ) primary sporidia are robust, long-cylindrical, and taper slightly toward both ends, but more so toward the apex (FIG. 1, A, B).

The number of primary sporidia on the promycelium apparently is fairly constant. In this study, the number ranged from three to eight, but five and six were predominant. The number and frequency of primary sporidia of 83 germinating spores were as follows:

Number of primary sporidia	3	4	5	6	7	8
Frequency	2	17	40	19	3	2

Apparently the number of primary sporidia observed is slightly higher than that reported by previous workers (**3**). Worthy of note is the fact that some germinating spores failed to form any primary sporidia. In such cases the promycelium kept elongating, and the protoplasm was localized in the apex and was separated from the proximal portion of the promycelium by the formation of consecutive septa. The promycelium might branch at the tip, in which case protoplasm flowed into the newly formed branches. Occasionally primary sporidia eventually were formed,

but the number of sporidia on such promycelia was always less than on the normal ones. In most cases, such promycelia eventually ruptured and died. From 10 to 90 percent of the germinating spores in any one plate may exhibit abnormal germination. The underlying factors involved have not been determined. Circumstantial evidence, however, indicates that the amount of moisture and oxygen surrounding the germinating spore may exert an influence. This supposition is supported by the fact that almost all the primary sporidia formed are projected from the surface of the plate into the air, indicating that aeration is an important factor.

Upon germination the primary sporidia of *Tilletia cerebrina* usually give rise to infection hyphae (FIG. 1, B), which, like the germination of fused pairs of primary sporidia in *T. caries*, keep elongating with protoplasm in the tip only, frequently becoming coiled and eventually dying. Only infrequently did the primary sporidia of *T. cerebrina* germinate by the formation of basidiospores. Great difficulty was, therefore, encountered in obtaining cultures of this fungus. After repeated attempts, it was found that when primary sporidia are grown in Petri dishes and adequate relative humidity is maintained, some of the sporidia give rise to basidiospores. The formation of new basidiospores by germination of previously formed basidiospores may be repeated a few times. These basidiospores were able to grow and form cultures which were microscopically indistinguishable from those of other *Tilletia* species.

No fusions have been observed between primary sporidia on artificial media. All attempts have failed to produce fusion between primary sporidia either from the same chlamydospore or from different ones. In this respect, the present species bears a close resemblance to *Tilletia elymi* (**5**).

CYTOLOGY

The thick, opaque character of the epispore has prevented observations of the early nuclear divisions within the spores. Subsequent nuclear divisions in the promycelium, which were believed to be mitotic in nature, resulted in a fairly large number of supposedly haploid nuclei (FIG. 2, A, B). The number of nuclei found in the promycelia varied from 14 to 32, depending on stages of growth. The most frequent number was 27. In most cases the somewhat angular contour of the nuclei suggested that they were at different stages of division. As shown in FIG. 2, B, 31 dividing nuclei apparently are visible in a segment of the promycelium. A deeply stained body located at each end of the dividing nucleus is suggestive of late anaphase.

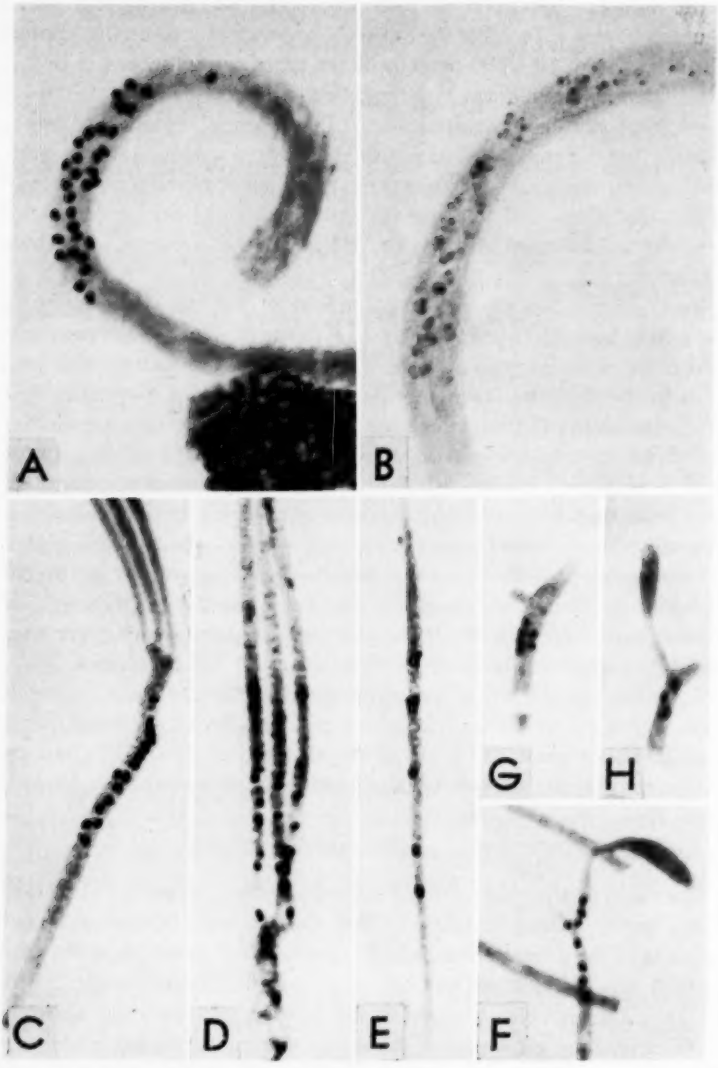


FIG. 2. Nuclear behavior in *Tilletia cerebrina*. A. A segment of promycelium with 32 nuclei. B. A segment of promycelium with 31 dividing nuclei apparently in the late anaphase. C, D. Successive stages of migration of nuclei from the mycelium in the formation of primary sporidia. E. A primary sporidium with 5 nuclei. F, G. Migration of nuclei in a germinating primary sporidium. H. A germinating basidiospore showing nuclear migration. All except B, × 1200. B, × 1840.

The formation of primary sporidia was accompanied by the flow of protoplasm and nuclei from the promycelium into the primary sporidia. The usual number of nuclei in the primary sporidium was four or five, rarely up to seven (FIG. 2, D–F). The nuclei in the primary sporidium apparently originate as a result of nuclear migration from the promycelium rather than as a result of later divisions of a single nucleus in the primary sporidium. This belief is supported by the observation that in most cases the number of nuclei in the promycelium was in accord with the total number of nuclei in the multinucleate sporidia. Furthermore, intermediate stages of nuclear migration during the formation of sporidia have been observed. For example, in FIG. 2, C, 14 nuclei are shown in the tip of a promycelium bearing three primary sporidia. In FIG. 2, D, apparently four to five nuclei have moved into the proximal portion of each of the three primary sporidia still attached to the promycelium. In this case one nucleus is still in the promycelium. If the dicaryophase is initiated in the primary sporidia apparently it is done by the association of nuclei which were formed in the promycelium. These primary sporidia should behave like those of a homothallic species, and not fuse, as in most other *Tilletia* species for which such phenomena have been described.

Nuclear migration also occurs during the germination of primary sporidia to form basidiospores. In FIG. 2, F, four nuclei are shown located in one end of a germinating primary sporidium while three others have moved into the sterigma. Presumably the resulting basidiospore would be multinucleate (FIG. 2, G, H). However, it is not certain whether it would have the same number of nuclei as its parent primary sporidium. Repeated germinations of this multinucleate basidiospore result in the formation of uninucleate basidiospores. It seems probable that nuclear dissociation takes place during this process, although just how is obscure.

DISCUSSION AND CONCLUSIONS

In most of the *Tilletia* species previously studied, the primary sporidia as well as secondary basidiospores were predominately uninucleate and haploid. Recently, Holton (**5**) reported that in *T. clymi* both primary and secondary sporidia (basidiospores) are multinucleate. The absence of sporidial fusions and the multinucleate condition of the primary sporidia were believed to be indications of homothallism in this species. In the present study, similar observations were made in *T. cerebrina*. The multinucleate condition of the primary sporidia of *T. cerebrina* originates by the nuclear migration from the promycelium rather than by divisions

of a single nucleus in the sporidium. Apparently these sporidia are dicaryotic because they do not fuse. On artificial media the primary sporidia usually germinate by infection hyphae, and occasionally produce multinucleate basidiospores, which, after repeated indirect germination, give rise to uninucleate basidiospores. The uninucleate basidiospores could be isolated and grown in culture, indicating that they are haploid. It can thus be assumed that the earlier dicaryotic nuclear association in the primary sporidia terminates during the course of repetitive basidiospore germination, and the later generations of basidiospores revert to the monocaryotic phase. It is of interest to note that in *Ustilago maydis*, a heterothallic species, diploid sporidia are known to occur (**1**). In contrast to the dicaryons of *T. cerebrina* and *T. elymi*, the solopathogenic lines of *U. maydis* are culturable. It appears that in some of the smut fungi, either heterothallic or homothallic departures from their normal life histories may occur, at least *in vitro*. The capacity of each species to develop in artificial culture apparently is determined by the nuclear conditions of the primary sporidia and basidiospores.

ACKNOWLEDGMENT

The writer wishes to express his sincere appreciation to Drs. C. S. Holton and George W. Fischer for advice, suggestions and criticism during the course of the work, and the supplying of materials used in this investigation.

LITERATURE CITED

1. **Christensen, J. J.** 1931. Studies on the genetics of *Ustilago zeae*. Phytopath. Ztschr. **4**: 129–188.
2. **Fischer, G. W.** 1953. Manual of the North American smut fungi. xii + 343 pp. The Ronald Press Co., New York.
3. —— **and Elisa Hirschhorn.** 1945. The Ustilaginales or "Smuts" of Washington. Wash. Agr. Expt. Sta. Bull. No. 459, 84 pp.
4. **Holton, C. S.** 1951. Methods and results of studies on heterothallism and hybridization in *Tilletia caries* and *T. foetida*. Phytopathology **41**: 511–521.
5. ——. 1953. *Tilletia elymi*, an apparently homothallic species. Phytopathology **42**: 635–636.
6. —— **and F. D. Heald.** 1941. Bunt or stinking smut of wheat. ii + 211 pp. Burgess Publishing Co., Minneapolis, Minn.
7. —— **and W. N. Siang.** 1953. Spore germination in *Tilletia asperifolia*, a species unique to the genus. Phytopathology **43**: 219–220.
8. ——, **R. H. Bamberg and R. W. Woodward.** 1949. Progress in the study of dwarf bunt of winter wheat in the Pacific Northwest. Phytopathology **39**: 986–1000.
9. **Liro, J. I.** 1938. Die Ustilagineen Finnlands II. Ann. Acad. Scient. Fenn., Ser. A. **42**: xiii + 720 pp.

A NEW SPECIES OF COMATRICHA FROM JAMAICA [1]

CONST. J. ALEXOPOULOS AND E. S. BENEKE

(WITH 2 FIGURES)

For the past few years the senior author and his students have been studying the species of Myxomycetes which develop in moist chamber culture on bark from living trees collected in Michigan and Florida. The genus *Comatricha* is generally well represented in such cultures, particularly by the minute species which appear to be common and widely distributed, but which are seldom collected in the field because they escape detection on account of their minute size.

Species of *Comatricha* which have developed in this manner in our laboratory include: *C. cornea, C. elegans, C. fimbriata, C. laxa, C. nigra, C. subcaespitosa, C. reticulata,* and an undescribed species from Florida (**1**).

During the summer of 1952 we spent several weeks on the island of Jamaica, B. W. I., collecting Myxomycetes. Specimens of bark from various living trees were also collected and placed in moist chamber. Among the slime molds developed in culture on bark, a species of *Comatricha* appeared which did not fit the description of any known species. Through the kindness of Dr. G. W. Martin of the State University of Iowa, who examined a slide preparation of our material and expressed the opinion that it probably represented an undescribed species, we examined authentically identified material of *C. fimbriata* and *C. cornea* which our species most closely resembles, and found our material to be distinctly different, particularly in the spore markings. We are, therefore, describing it as a new species under the name *Comatricha martinii* in honor of Dr. G. W. Martin of the State University of Iowa.

Comatricha martinii sp. nov.

Sporangia solitaria, erecta, brunnea, 0.5–0.6 mm. alta, 0.08–0.15 mm. diam., stipitata, stipite brunneo, 0.45–0.5 mm. alto, in sporangium tamquam columella penetrante et ad centrum attingente; capillitium ex apice columellae enascens, e 3 aut 4

[1] Contribution No. 53–11 from the Dept. of Botany and Plant Pathology, Michigan State College, East Lansing, Michigan. Published out of order at expense of Department of Botany, Michigan State College.

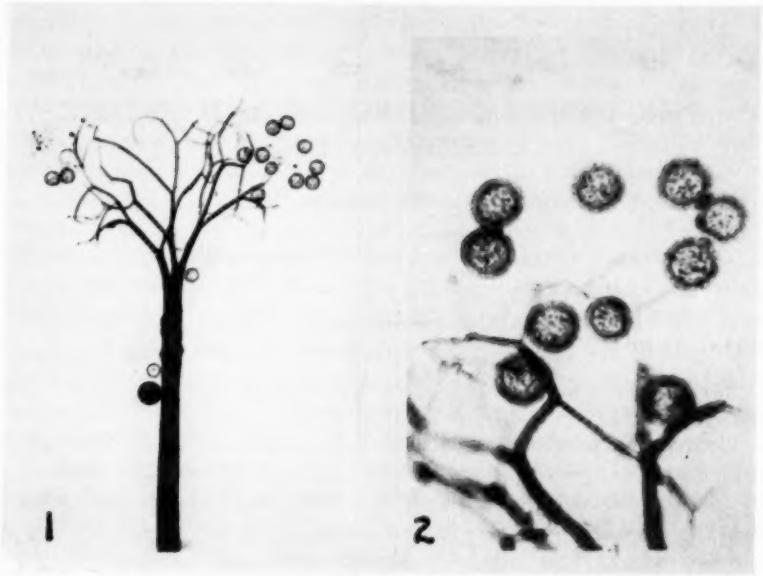
ramis tenuibus, dichotomo-furcatis, brunneis consistens; sporae globosae, violaceo-cinereae, verrucosae, verrucarum circulos dispersos, prominentes ferentes, 6.5–7 μ diam.

Sporangia scattered or solitary, brown, globose, stipitate, minute, 0.08–0.15 mm. in diameter, their total height 0.5–0.6 mm.; peridium completely evanescent; stalk slender, tapering upward, and continuing to the middle of the sporangium as a columella; capillitium lax, arising from the tip of the columella, consisting of three or four slender, brown branches which fork dichotomously three or four times with no anastomoses; spores brown in mass, violet-gray by transmitted light, minutely verrucose, bearing scattered, prominent clusters of warts, 6.5–7 μ in diameter.

Type developed in moist chamber culture on bark of living unidentified tree, collected on the property of Eaton Hall Hotel, Runaway Bay, St. Ann, Jamaica, B. W. I., July 26, 1952; deposited in the Mycological Collection, Michigan State College Herbarium, Myxomycetes of Jamaica No. 78. The type consisted originally of about 25 well developed sporangia all but six of which disintegrated by the time the specimens reached our laboratory at East Lansing, leaving only their stalks as evidence of their development. In order to avoid complete loss of the type material, three of the remaining sporangia have been mounted on slides in Hoyer's medium for permanent preservation. One of these slides remains at Michigan State College, one has been sent to the Herbarium of the State University of Iowa, and one to the Herbarium of the New York Botanical Garden.

The distinguishing features of *Comatricha martinii* are its minute size, the lax capillitium, and the prominent clusters of warts exhibited by the minutely verrucose spores. The globose sporangium with lax capillitium places it close to *C. cornea* and *C. fimbriata*. It differs, however, from the former in that it lacks the translucent stalk and the small collar at the base of the sporangium, and from both in the character and size of its spores. *C. typhoides* is the only other known species of *Comatricha* with spores bearing clusters of warts. The sporangia of that species, however, are cylindrical rather than globose, have intricate rather than lax capillitium, and reach a total height of 2–5 mm whereas those of *C. martinii* are scarcely 0.6 mm high.

We wish to express our appreciation to Dr. C. B. Lewis, Director of the Institute of Jamaica, Kingston, and to Prof. G. F. Asprey of the Department of Botany, University College of the West Indies, Mona, for laboratory facilities extended to us during our stay in Jamaica; to Dr. G. W. Martin of the State University of Iowa for examining our material and lending us material for comparison; to Dr. E. A. Bessey



FIGS. 1, 2. *Comatricha martinii*. 1. Photomicrograph of entire fructification with spores removed to show origin and nature of capillitium. × 260. 2. Photomicrograph of spores under oil immersion objective, showing clusters of warts on the surface. × 1140.

of Michigan State College for correcting the Latin diagnosis; and to Mr. Philip G. Coleman of Michigan State College for preparing the photomicrographs. The cost of excess pagination, to enable this article to appear in an early issue of MYCOLOGIA, was borne by the Department of Botany, Michigan State College, and our thanks go to Dr. William B. Drew, head, for making this possible.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY,
MICHIGAN STATE COLLEGE,
EAST LANSING, MICHIGAN

LITERATURE CITED

1. **Alexopoulos, Const. J.** Myxomycetes developed in moist chamber culture on bark from living Florida trees; with notes on an undescribed species of *Comatricha*. Quart. Jour. Fla. Acad. Sci. (In Press).

THE GENUS CORDYCEPS AND FRIES'S OBSERVATIONES

DONALD P. ROGERS

The well-known fungus genus *Cordyceps* has an earlier name, *Cordylia,* given to it by Elias Fries. During work on nomina generica conservanda proposita the following list of synonyms and citations was hunted down and compiled:

Cordylia Fr., Obs. Mycol. **2**: 316. 1818.
Cordyceps Fr., Obs. Mycol. **2**: 316. 1818.
Cordylia Fr. ex Ficinus & Schubert, Fl. Dresden 2 ed. **2**: 331. 1823.
Sphaeria ser. I [sect.] *Cordyliae* Fr., Myk. Hefte **2**: [33]. 1823.
Sphaeria sect. I *Peripherica*e Trib. *Cordyceps* [Fr.] Fr., Syst. Mycol. **2** (2): 320, 323. 1823.
Cordyceps Link, Handb. Gew. **3**: 346. 1833.

A number of points in this synonymy deserve comment. One is the authority given for the generic name *Cordyceps* as first validly published: Link (1833) does not cite Fries as author of the name, and in consequence there probably is no occasion for writing "(Fr.) Link." (Cf. Art. 59 and examples.) There is only one difference in the treatment of the name by these two authors, as already noted (Rogers, Farlowia **3**: 438. 1949): *Cordyceps* as used by Link is masculine, whereas when Fries returned, in Summ. Veg. Scand. 381. 1849, to his earlier opinion that the group deserved generic rank, he treated the name as feminine. Since *Cordyceps* is adjectival in form, there is no philological reason for preferring one to the other; and under what seems to be the applicable provision of the code of nomenclature, Rec. 83A (3), specific epithets in later combinations should be made to agree in gender with Link's names.

The second point is that the retention of *Cordyceps* is not inhibited by the existence of the earlier synonym *Cordylia* Fr. ex Ficinus & Schubert 1823; for the latter is a homonym of *Cordylia* Pers., Syn. Plant. **2**: 260. 1807. In actual history, *Cordylia* Pers. is quite certainly a misspelling of *Cordyla* Lour., Fl. Cochinch. **2**: 411. 1790, and as such would have only the nomenclatural validity pertaining to any misspelling. But in publishing *Cordylia* Persoon did not attribute that name to anyone, and therefore *Cordylia* Pers. stands as a validly published name and a legal

barrier to the disinterment of *Cordylia* Fr. ex Ficinus & Schubert. It is also possible that *Cordyla* Lour. is itself an earlier homonym of *Cordylia* Fr. (Cf. Art. 82, examples of orthographic variants.)

The third point is that, in apparent refutation of the axiom that two things cannot occupy the same space at the same time, Fries described both *Cordylia* and *Cordyceps* in Obs. Mycol. **2**: 316. Here again is exemplified the extraordinary nature and history of Fries's *Observationes.*

Some years ago it was shown (Mycologia **31**: 297–307. 1939) that the pretended "edition nova" of the *Observationes Mycologicae* is no more than a reissue of the original (1815–18) pages with a new title-page, dated 1824, inserted. Conclusive evidence for this assertion was found in the fact that volumes bearing the two dates are identical not only in text, not only in spelling and type-face, but in the numerous irregularities—broken types, uneven lines, weak impressions—occurring throughout the work. The search for the first place of publication of *Cordyceps* and *Cordylia* brought out another irregularity in Fries's publication: that several pages of vol. 2 ("pars secunda") were revised after having been printed, and that copies now in existence differ in including one or the other of these sets of pages. The difficulty entailed was apparent from the reference to *Cordylia* in Pfeiffer's *Nomenclator* and in the Ficinus & Schubert *Flora der Gegend um Dresden, 2* ed., already cited; when two copies of the *Observationes* were consulted for a description of *Cordylia,* said by these authors to appear therein, the only account found appeared under the name *Cordyceps.* A third copy, however, one with the 1824 title-page and recently acquired for the library of The New York Botanical Garden, gave instead the name *Cordylia.* A comparison with the volumes reading *"Cordyceps"* brought out the following difference in names published in vol. 2:

	Original text (cancelland)	Revised text (cancellans)
p. 15	Obs. on *Agaricus Hornemanni; A. reflexus.*	*A. Mülleri; A. rhabarbarinus.*
p. 16	discussion of *A. reflexus.*	*A. reflexus.*
p. 249	Sectio 1. Stipite annulato.—Trib. I *Annulati;* Sect. 2. Sripite [!] cortinato.—Trib. II. *Cortinati;* Sect. 3. Stipite squamoso. Trib. III *Asperati;* Sect. 4; Sect. 5.	Trib. I. *Annulati* [centered over Sect. 1]; Trib. II. *Cortinati* [centered over Sect. 2]; Trib. III. *Asperati* [centered over Sect. 3; numbers 4 & 5 for species, not sections, under this Trib. & Sect.]

	Original text (cancelland)	Revised text (cancellans)
p. 250	Sect. 6; Sect. 7; Trib. IV. *Luridescentes;* Sect. 8; Sect. 9; Sect. 4. Stipite reticulato.	[6, 7, 8, & 9 are numbers for species; Trib. IV & Sect. 4 stand.]
p. 309	V. Dermea; Peziza.	[Same names; but text shortened to admit the following:] Sub Peziza plura latent genera. Typus novi generis, disco farinoso distincti, est *Peziza coriacea* Bull. t. 438. f. 1 Haec stipitata est & *Heyderia cinerascens* Mihi; aliam sessilem *Heyd. testaceam* dico. Utraque fimicola, Ascoboli instar, habitu Poroniae.
p. 310	Helotium P.; H. aciculare.	Ascobolus; A. pilosus; A. aeruginеus; Helotium Pers. nec Tode; H. subtile.
p. 311	H. subtile.	H. phasoides; Acrospermum; A. micans; A. Caliciforme.
p. 312	Phacidium; P. lacerum; P. sphaerioides.	A. fluxile; Scleromyci; Phacidium; [list, with basonyms, only:] P. Ilicis, Taxi, leptideum, infractum, repandum.
p. 313	Fungi cupulares (*Peziza, Helotium, Ascabolus* [!] et *Phacidium*) varia adhuc continet nova Genera, fructificationis processu diversa. . . . *Pezizam coriaceam Bull.* p. 258. t. 438 f. 1. . . . non propriam tantum esse speciem; set etiam Genus disco pulverulento distinctissimum. Est igitur STERREBEELLIA *cinerascens* Mihi stipitata et aliam in Scania, locis similibus, legi, quae *Sterrebeellia testacea. Mihi.* sessilis.	[listing continued:] Pini, Ledi, testudineum, costatum, angulatum n. sp., umbonat. n. sp.; [descr.:] P. lacerum.
p. 314	Prodromus novae dispositionis Sphaerioideorum; Poronia Fr. (beat. Willdenow nominis auctor).	Poronia Willd. Fries.

	Original text (cancelland)	Revised text (cancellans)
p. 315	P. truncata; P. repanda.	P. truncata; P. repanda.
p. 316	Cordylia; I. Creatocaulon (carnosae).	Cordyceps; d. [!] Hypocreas (carnosae).

Since the 1824 title-page was prepared only for vol. 1, both of these versions appear in copies of vol. 2 bearing the same title-page dated 1818. As already noted, the original text is found bound with a copy of vol. 1 marked "Edition nova. 1824"; the revised text is bound with the 1815 title-page for vol. 1. If the history of the two "editions" were not known, the anomalous phenomenon would be encountered of a first edition revising a second. Nevertheless, the mutual relation of the two versions can be readily established: (1) In one version pp. 15–16 are printed on the same sheet of paper as pp. 1–2, but in the other pp. 15–16 are on a separate sheet glued into place; and pp. 249–50 have the same relation to pp. 247–8; in each place the separate sheet is presumably the cancellans, and is so marked here. (2) Obvious errors in the one version, like the numbering of species as sections on p. 249, are corrected in the other. (3) In El. **2**: 54, under Trib. I. *Cordyceps*, Fries wrote: "Obs. *Cordyliam* l.c. [Syst. Mycol. **2**: 323] non dixi ob genus phanerogamum homonymon Loureiro." Therefore *Cordylia* is the original name and *Cordyceps* the substitute. So far as the copies at hand are concerned, then, it is those bearing the date 1815 that include Fries's definitive text for the differing pages; the "second edition" at hand was made up from left-over uncorrected sheets. From their dates, of course, none of the names in either the revised version or the original is validly published; but all are effectively published; and when they are referred to in later works, the fact that some names may be absent from the copy used can occasion more than a little trouble. Fries, for example, in Syst. Mycol. **2**: 158, refers to *Patellaria* Fr. as "Nov. genus. *Fries Obs. 2. p.* 313," a reference to the original version, where the new genus is called *Sterrebeellia*. There is no mention of the revised version, where the same genus is described on p. 309 and called *Heyderia*. On the other hand, in Syst. Mycol. **2**: 323 Fries refers to *Cordyceps* as published in Obs. **2**, p. 316 (revised version), neglecting *Cordylia* in the original.

Whatever the rank or composition of *Cordyceps* and *Cordylia*, it is clear that *C. militaris* is, ll. cc., the first and most completely described species, and the type of the genus. Under the new Art. 23f the correct citation of the basonym, as of a number of others, may at first seem a matter of some uncertainty. The species is called *Sphaeria militaris*

[L.] in Fries, Syst. Mycol. **2** (2) : 323. 1823; it is also *S. militaris* [L.] in Purton, App. Midl. Fl. **1**: 276. 1821. The Rule provides (a) that the starting-point for the nomenclature of such fungi is January 1, 1821, and (b) that names published elsewhere between that date and the date of vol. 3 (2) of the *Systema* "which are synonyms or homonyms of names of any of the Fungi caeteri included in the *Systema* do not affect the nomenclatural status of names used by Fries in this work." That means, of course, that if Purton had called the species *S. quaelibet*, Fries's name would stand. But Purton's use of *S. militaris* for the same fungus does not conflict with Fries's; and since *S. militaris* [L.] Purton is apparently the first publication of that binomial after the starting-point, the Purton citation is presumably the correct one for the combination. *Hypoxylon militare* [L.] Mérat, Nouv. Fl. Paris 2 ed. **1**: 137. 1821, is, however, earlier than either; for Mérat appeared in the week of June 9–15 (cf. Mycologia **43**: 377), and Purton's part 1 after August 16 (cf. App. Midl. Fl. **1**: viii) and probably after September 20 (op. cit. **2**: 574, footnote b), or even in the month ending December 1 (on which date it is listed under "New Publications" in the New Monthly Magazine and Literary Journal [London] [II] **3**: 625; but see comment in Mycologia **43**: 378 for the listing of Gray in that periodical). Since it is not the binomial *S. militaris*, but only the epithet, that is in question as one seeks the best citation for the type of *Cordyceps*, it appears to the writer that the genotype should be cited as *H. militare* [L.] Mérat, l.c.

To turn again to Fries's book, a complete copy of the *Observationes* should include the following pages, bound in the order indicated:

(1) Title-page; (a) original, dated 1815, and reading "auctore E. M. Fries"; or (b) later, dated 1815, reading "auctore Elia Fries," and bearing the line "Pars prima"; or (c) "edition nova," dated 1824.
(2) A page of dedication, a page promising a second part, and 3 pp. of "errata graviora." These form a 4-leaf gathering with the later 1815 title-page, and therefore should not be found in copies with the original title-page.
(3) Text, pp. [1]–230.
(4) Plates I–IV, colored.
(5) Title-page for "pars secunda," dated 1818.
(6) Preface ("Lectori benevolo S."), pp. [III]–X.
(7) Text, pp. [1]–372.
(8) Addenda, pp. 373–6.
(9) Explicatio iconum (Pl. 5–8).
(10) Plates V–VIII, colored.

When the nature and status of the so-called new edition were earlier discussed, only one copy, that of the Massachusetts Horticultural Society, had been examined; a brief description and several photographed pages of a second, in the Lloyd Library, furnished additional information. Since then a description of a third copy, at Cornell University, was furnished by Dr. H. M. Fitzpatrick; a fourth, in the University of Chicago Library, was found by Sister Mary Cecilia Bodman and lent by the library; and a fifth was purchased by the Garden. Of these and the copies with an 1815 title-page that have been carefully examined no two are alike, and none is complete. In the earlier discussion it was observed that whoever had taken the trouble to create a spurious second edition by the insertion of the 1824 title-page had been somewhat careless in leaving the original (1818) title-page of the second volume unaltered. In the Cornell copy there was no such oversight; the title-page for vol. 2 is omitted, and the preface to that volume is bound in at the beginning of vol. 1. In both the Chicago and the N. Y. B. G. copies, on the other hand, not only the title-page for vol. 2, but the original title-page for vol. 1 is retained, the "edition nova" page being merely inserted directly before the latter. These copies then provide manifest confirmation of the thesis earlier defended at considerable pains, that the new edition is only a reissue.

THE NEW YORK BOTANICAL GARDEN,
BRONX PARK, NEW YORK 58, N. Y.

NOTES AND BRIEF ARTICLES

Heterothallic behavior in the Aspergillaceae

Recently, Raper and Fennell (Mycologia **44**: 101. 1952) in a report entitled "Homothallism vs. heterothallism in the *Penicillium luteum* series" stated that after much experimentation with a number of isolates, they were unable to corroborate the earlier report of Derx (Trans. Brit. Myc. Soc. **11**: 108. 1926) on the existence of heterothallism in *P. luteum*, nor were they able to demonstrate heterothallism in any related species. In their discussion these authors suggested, as one possibility, the existence of both homothallic and heterothallic behavior in the same species. In view of certain other investigations it appears that this might well be the key to this puzzling situation.

Henrard (La Cellule **43**: 351. 1934), whose interesting publication has been overlooked by most investigators in this field, demonstrated in *Aspergillus nidulans* a phenomenon which he called "physiological heterothallism"—a term of somewhat doubtful value. He discovered in his material two different strains which, when paired, produced a red pigment along the line of contact. Although each strain was self-fertile, cross-karyogamy occurred when the two were paired, as evidenced by the fact that both types of ascospores could be isolated from cleistothecia produced at the line of contact.

Fifteen years later, Pontecorvo *et al.* (Heredity **3**: 385. 1949), experimenting with biochemical and morphological mutants in *A. nidulans*, were able to synthesize heterokaryons from cultures differing in a number of characters. In an analysis of asci produced by these heterokaryons, they showed that cross-karyogamy had occurred in many of the cleistothecia, self-karyogamy in others. Hemmons *et al.* (Heredity **6**: 135. 1952), studying 22 different mutant strains of the fungus, confirmed this report and further discovered that in 10 out of 22 crosses, the proportion of asci showing cross-karyogamy in the area of contact between paired cultures approached 100%. Since most of the strains used were self-fertile, the term "relative heterothallism" was applied to this situation to imply the existence of preferential cross-karyogamy.

In view of these findings it seems logical to conclude that Derx had observed much earlier a somewhat similar, though more highly modified, condition in *Penicillium luteum*. From his original fertile culture he

obtained single-ascospore cultures, differing in growth characters and in conidial color, which were self-sterile but which produced fertile perithecia in appropriate crosses. The evidence presented by Derx indicates that his original isolate was heterokaryotic, and that various segregants involving conidial color, certain growth characters, and mating reactions were recovered in the single-ascospore isolates. It is unfortunate that Derx made no genetic analysis of the crosses, since this would have thrown much light upon the nature of the factors controlling the mating reactions. However, the data on the relative inter-fertility of the various strains indicate that the mating reactions may be controlled at two or more loci, with the possible involvement of some multiple allelomorphic factors. It is obvious that the heterothallic tendencies described by Derx are more highly developed than those described by the other investigators mentioned, for his single-spore cultures were no longer self-fertile, although the isolates that reacted most vigorously in crosses were those that produced sterile cleistothecia when grown singly.

These behavior patterns, which indicate the existence of homothallic and heterothallic tendencies in the same species, are not unique to the Aspergillaceae. Related phenomena have been described in certain yeasts, *Hypomyces solani, Glomerella cingulata, Ophiostoma fimbriata, Sordaria fimicola* and others. Perhaps the definition of the term "amphithallism" proposed by Lange (Dansk. Bot. Ark. **14**: 1. 1952) for certain Basidiomycetes could be extended to include these fungi also. The evidence indicates that it is not wise to conclude that any fungus is strictly or irrevocably homothallic. It would also appear that heterothallic tendencies in homothallic fungi are primarily the means by which such fungi may outbreed, very much as in heterothallic fungi.—LINDSAY S. OLIVE.

LEPTODISCUS TERRESTRIS: A CORRECTION

In the paper on root rot of legumes in the July–August 1953 issue (**45**: 548–554), the first sentence of the last paragraph on p. 549 should read: "The radial stroma often bears a superficial resemblance to the scutellum of members of the Microthyriaceae," rather than Micropeltaceae as printed.—J. W. GERDEMAN.

MEMOIR SERIES

The Mycological Society of America and the New York Botanical Garden announce joint sponsorship of a memoirs series and are now in a position to initiate such a series. Each memoir will be sold separately and continuing subscriptions to the series will be accepted, independently

of purchasers' membership in the Mycological Society or subscription to MYCOLOGIA. The following stipulations should be noted by prospective contributors:

1. Papers may vary in length from approximately 75 to a maximum of 200 printed pages.
2. Authors must have maintained membership in the Mycological Society of America for at least one year prior to the submittal of a manuscript.
3. Any paper of a mycological nature and representing a comprehensive treatment of a subject will be considered.

Since the publications in this series are to be self-liquidating, preference will necessarily be given to contributions of wide interest. The committee listed below invites preliminary communications from Society members who may have ready or in preparation manuscripts of suitable content and size.

L. S. WICKERHAM
G. B. CUMMINS
M. P. BACKUS, Chm.

REVIEWS

LA DÉSCRIPTION DES CHAMPIGNONS SUPÉRIEURS, by Marcel Josserand. (Encyclopédie Mycologique, Vol. XXI.) 338 pp., 232 figs. Paul Lechevalier, Paris. 1953. Price 3500 fcs. (about $10.00).

This book is exactly what the title indicates—a treatise on the technique of describing the higher fungi. It is a pleasure to review a book which accomplishes its objective in the fine style of this one. We all know the author as an investigator who is a master of the art of both precise description and accurate illustration, and it is only natural that he should recognize the need for a standard of descriptive procedure for the higher fungi, and that he would do something about it. I hope that it will be translated into a number of languages so that it can be made available to amateurs the world over. It is just as valuable for professional mycologists, but they should be able to read the original version.

The book consists of two parts: the first is a discussion of descriptive technique and problems related to it; the second is a glossary of descriptive terms. It is an ideal work for graduate students to study for their requirement of the French language. For, rather than give just a brief definition of a term, Josserand has given, in cases where it was pertinent, a discussion which is very clarifying. One very fine feature of this book is that the author has not been dogmatic as to procedure. In the matter of making free-hand sections he illustrates two methods with photographs. Your reviewer has a very strong preference for the one shown on plate 2, p. 73, but with an old fashioned hollow ground straight-edge razor as a sectioning instrument instead of a safety razor blade. Every man who has developed his own techniques, as most of us have, will find that there are minor differences in the way we do some things to the way Josserand suggests, but the significant feature is that the author has always kept what a given technique *was designed to accomplish,* or a term to *describe,* foremost in mind. Thus, in a work where there would naturally be a strong tendency to fall into a dogmatic pattern, the author has remained above it. To me this is the highest compliment that can be given to a book of this type.—ALEXANDER H. SMITH.

THE USTILAGINALES OF THE WORLD, by George Lorenzo Zundel. Contribution No. 176 from the Department of Botany, The Pennsylvania State University, School of Agriculture. xi + 410 pp., 3 portraits. (Multilithed.) 1953. Price $3.50.

The manuscript for this book was completed in 1945. Its posthumous appearance at this time represents final victory over a long succession

of frustrated attempts to get the voluminous manuscript (nearly 1000 pages) published. War conditions, which prevailed at the time the manuscript was completed, were responsible for the first frustrations in effecting publication. Following were a succession of insurmountable difficulties in obtaining letter press printing of the manuscript. Its eventual publication by the multilith process by the Pennsylvania Agricultural Experiment Station, while not as attractive as letter press printing, is equally as usable, especially considering the fact that not a single smut is illustrated.

"The Ustilaginales of the World" is primarily a compilation of descriptions and pertinent records of species of the smut fungi. Although in the introduction the author claims, "the concept of species as used in this paper is very broad," the species concept seems narrow to this reviewer. Only rarely are there critical comparisons of species, and, on the basis of the descriptions, synonymous relationships abound among the species included. There is a key to the two families recognized and a key to the genera, but no keys to species. For the larger genera the preparation of keys to species would have been a superhuman task, considering the great morphological similarity among many of the species included in these genera.

The introduction contains a good historical account of smut diseases, with shorter treatments dealing with the nature of smut fungi, their taxonomy and cytology. This section is concluded with 52 selected references.

In the taxonomic section, which constitutes the major part of the book, the genera are taken up in alphabetical order, those of the Ustilaginaceae being treated first. Each genus and each species is represented by citation of authority, place of publication, synonyms with their place of publication, type, type host and locality, a formal description, hosts and geographic distribution by host. Brief notes are included for some of the species. Well over 1200 species and varieties in thirty genera are thus treated. The section is concluded with a complete host index to the species and an index of synonyms and of smut species mentioned other than in the normal alphabetical order. The host index does not appear to have reconciled host synonymy. For example, it includes *Agropyron trachycaulum*, *A. tenerus*, and *A. pauciflorum*, with smut species indexed under each.

In the foreword it is explained that, "the manuscript is being presented here as nearly as possible in the final form in which Dr. Zundel left it and practically without corrections and additions," and that "It was felt that since Dr. Zundel had presented it as ready for publication it should

be put out unchanged as a contribution by him as of the year 1945." This accounts for the unfortunate fact that appropriate portions of the manuscript were not changed to conform with certain portions already selected out by Dr. Lee Ling [1] and published in Zundel's name. Thus it is that several binomials already published still appear in "The Ustilaginales of the World" as "n. sp." or "comb. nov." This is apt to result in variable citation of these species and combinations.

There are numerous errors (mostly minor) throughout the book. For example, authority for *Ustilago williamsii* is given as "(Griff.) G. W. Fischer and Elisa Hirshhorn, Mycologia **37**: 253. 1945," where it should read (Griff.) Lavrov, Trav. Inst. Sci. Biol. Univ. Tomsk **2**: 22. 1936. It was Dr. Zundel himself, in 1944, who called my attention to the fact that Lavrov had already made this combination. Why he cites the authority he does is a mystery. Likewise, he includes both *Urocystis fraseri* Clint. and Zundel and *Sorosporium granulosum* Ellis and Tracy, whereas actually these binomials refer to one and the same smut. In the introduction it is stated that, "the violet (Viola sp.), . . ." is "attacked by . . . *Urocystis cepulae*." Undoubtedly this was intended to be *Urocystis violae*. And, on page 11, it is stated that Christensen and Rodenhiser presented an extensive review on the physiologic specialization and genetics of the smut fungi in *1904!*

None of the smuts is illustrated in the book but there are three portraits: Dr. Zundel's paternal grandfather, James Ingram, on whose farm he first observed smut and with whom he first discussed the nature of smut; G. P. Clinton, under whom Dr. Zundel studied for the doctorate at Yale University; and a portrait of Dr. Zundel himself, included by the publishers.

Anyone who has ever written any kind of scientific book knows how easy it is for errors to creep into the manuscript and survive subsequent editing, and thus he tends to take a sympathetic view of such errors in the works of his colleagues. There is no doubt in my mind but what this book, in spite of numerous minor errors and in spite of now being eight years out of date, is truly a valuable and useful register of the smut fungi from all over the world, especially in view of the modest price ($3.50 plus $.50 handling charges). Orders should be sent to the Pennsylvania Agricultural Experiment Station, Room 107, Agriculture Building. Checks should be made payable to the Pennsylvania State University.—GEORGE W. FISCHER.

[1] Zundel, George L. Notes on the Ustilaginales of the World. V. Mycologia **43**: 267–270. 1951.

DISPERSAL IN FUNGI, by C. T. Ingold. viii + 197 pp., 90 figs. Clarenden Press, Oxford. 1953. Price $3.50.

Few aspects of mycology are more fascinating than that which embraces the diverse and efficient mechanisms developed by fungi in connection with spore liberation and dispersal. Although Dr. Ingold states in the preface to this small but most valuable and interesting book it is not designed to meet the needs of any particular group of biologists, all who are concerned with fungi will derive much profit from reading it whether their interest lies in mycology in the strictest sense or in some applied science.

The style is simple and direct, the explanations clear and concise. The book is amply illustrated, mostly by well-reproduced line drawings. In a few places, the legends for drawings are printed in type which is too small for comfortable reading. The printing conforms to the usual high standard of the Oxford University Press. Only one typographical error was detected.

In seven chapters, the book deals with the following topics: spore liberation, the occurrence and distribution of spores in the air; spore dispersal by insects, by larger animals and by water; the dispersal of fungi on and in seeds.

One might question the use of the term "shadow yeasts" (p. 70) to apply to the Sporobolomycetaceae, because they are not considered yeasts by most mycologists (Bulat has reported recently that *Dacrymyces Ellisii* has an imperfect stage producing ballistospores characteristic of the Sporobolomycetaceae). Moreover, the word "shadow" will be appreciated only by mycologists who have cultured these fungi.

Recognizing that germ tubes as well as zoospores may be developed by the germinating asexually produced spores of *Phytophthora infestans*, it is questionable whether the term "conidium" should be used (p. 9) rather than "sporangium."

In view of Wright's report of germination of spores of *Itajahya galericulata*, the statement that spores of the Phallaceae have never been observed to germinate (p. 120) might have been modified.

Complete coverage is not to be expected within the limits of a small volume. However, one would have liked to see some reference to the discharge of the teliospores of *Puccinia tumidipes* through the action of the hygroscopic pedicels as described by Pady and to the diurnal periodicity of ascus discharge in *Taphrina deformans* as reported by Yarwood.

The criticisms noted are decidedly of a minor character. Dr. Ingold has long been one of the foremost contributors to our knowledge of

spore discharge and dispersal in fungi and is to be congratulated on presenting a well rounded, authoritative and stimulating discussion of this important topic.—HAROLD J. BRODIE.

CENSUS CATALOGUE OF BRITISH LICHENS, by W. Watson. xix + 91 pp. Published under the auspices of the Foray Committee of the British Mycological Society. Cambridge University Press, London. Price sh. 7/6.

This check-list of British lichens has appeared after more than thirty years of study of previously published lists and of the specimens upon which the records were based. The specific identities are based on Miss A. L. Smith's "Monograph of British Lichens" (1911, 1926), except for a few genera for which extensive monographs have been published since. The arrangement of families and genera follows that of Watson (1930). A few new combinations are made, mostly from recognizing *Biatora* as distinct from *Lecidea*. For sources of information, there is a long list of collections and collectors and a bibliography of more than seven pages, but apparently Thomas Taylor's collections at the Farlow herbarium were not consulted for Irish localities. Each county and vice-county is given a number, enabling one to quickly visualize the recorded distribution in the British Isles. Locality symbols for doubtful records are enclosed in parentheses while square brackets indicate an old record and that the species is now probably extinct in that county or vice-county. Probably the geographic distribution of lichens in no other area of similar size is now so accurately known as that of the British Isles.—CARROLL W. DODGE.

MANUSCRIPT

Publication in MYCOLOGIA is ordinarily restricted to members of the Mycological Society of America. Exceptions to this regulation require a favorable vote by a majority of the Editorial Board. When a paper has two or more authors, the person submitting the paper is expected to be a member.

Papers should be submitted in duplicate, typwritten and *double-spaced throughout*, to any member of the Editorial Board, preferably to that member most familiar with the subject matter. Papers will be published in the approximate order of their acceptance, except for the address of the retiring President and papers whose cost of publication is paid by the authors, the latter run as excess pagination.

All illustrations should be numbered consecutively throughout a paper, using arabic numbers and small letters for subdivisions, e.g., Fig. 1, a etc. This does not mean that all figures grouped for convenience on a single page need have a single number. Figures should be prepared so that, when reduced, the width will not exceed 4 inches, and should be short enough to permit the insertion of the legend beneath the figures. Each article will be restricted to twenty pages, including illustrations, except when authors submit only one paper in two or three years of membership, in which case the restriction will be thirty and forty pages respectively. Ruled tabular matter is counted double. Should an author wish to publish additional pages in one article he may do so by paying for the excess pages at current rates.

Citations of literature should be double-spaced, arranged in alphabetical order and cited by numbers. In citing papers with two or more authors, only the first author should have the initials after the surname. The address of the author should appear at the end of the text, before the bibliography.

Each author will be restricted to two pages of half-tone illustrations for each article, or their equivalent (the cost of each being approximately $9.25). Should the author submit illustrations for which the cost of cuts exceeds that amount, he will be asked to bear the excess cost of the cuts in addition to excess pages.

To comply with the International Rules, it is recommended that contributors furnish *brief* Latin diagnoses of all new species and genera when their manuscript is submitted for publication.

PRICE LIST OF REPRINTS

	4pp. 1 to 4	8pp. 5 to 8	12pp. 9 to 12	16pp. 13 to 16	20pp. 17 to 20	24pp. 21 to 24	28pp. 25 to 28	32pp. 29 to 32
50 Copies	$3.93	$6.28	$9.81	$10.21	$13.35	$15.31	$17.66	$18.84
100 Copies	4.71	7.46	11.38	12.56	16.09	18.84	21.98	23.55
Additional Copies per C. .	1.57	2.36	3.93	4.71	5.50	7.07	8.64	9.42

For 500 copies deduct 5%; for 1000 copies or more deduct 10%.

Covers: For first 50 covers, $4.30; additional $3.10 per C.

For more than 32 pages add cost per schedule to make total. Example: for 44 pages add cost for 32 pages and 12 pages.

Note: For any reprints requiring additional composition or changes, either in text or cover, an extra charge will be made.

LANCASTER PRESS, INC.
LANCASTER, PA.

Partial List of Publications of The New York Botanical Garden

Mycologia, bimonthly; devoted to fungi, including lichens; containing technical articles and news and notes of general interest. $8.50 a year; single copies $1.75 each.

Established by The New York Botanical Garden in 1909, in continuation of the Journal of Mycology, founded by W. A. Kellerman, J. B. Ellis, and B. M. Everhart in 1885. Edited by William Alphonso Murrill, 1909–1924. Edited by Fred Jay Seaver, 1924–1946; by Alexander H. Smith, 1946–1950. Beginning with January, 1933, the official organ of the Mycological Society of America.

North American Flora. Descriptions of the wild plants of North America, including Greenland, the West Indies, and Central America. Planned to be completed in 34 volumes. Roy. 8vo. Each volume to consist of four or more parts. [Not offered in exchange.] Volumes 1–10 devoted to fungi.

Vol. 1, part 1, 1949. Myxomycetes. $7.25.

Vol. 2, part 1, 1937. Blastocladiaceae, Monoblepharidaceae, Saprolegniaceae, Ectrogellaceae, Leptomitaceae. $2.00.

Vol. 3, part 1, 1910. Nectriaceae-Fimetariaceae. $2.00. (Out of print.)

Vol. 6, part 1, 1922. Phyllostictaceae (pars). $2.00.

Vol. 7 (now complete), parts 1–15, 1906–1940. Ustilaginaceae-Aecidiaceae. $2.00 per part. (Parts 1–5 out of print.)

Vol. 9 (now complete), parts 1–7, 1907–1916. Polyporaceae-Agaricaceae (pars). $2.00 per part. (Parts 1–3 out of print.)

Vol. 10, part 1, 1914; parts 2 and 3, 1917; part 4, 1924; part 5, 1932. Agaricaceae (pars). $2.00 per part.

The New Britton and Brown Illustrated Flora of the Northeastern United States and Adjacent Canada. By Henry A. Gleason. 3 volumes. List price $30.00 per set; shipping charge $0.50. Succeeds the Illustrated Flora by Nathaniel L. Britton and Addison Brown. Includes descriptions and drawings of the plant species, from ferns to orchids, which grow without cultivation in the area extending from the St. Lawrence River to Virginia and westward to Missouri and Minnesota.

The Garden Journal of The New York Botanical Garden. Bimonthly, illustrated, containing news, book reviews, and non-technical articles on botany, exploration, and horticulture. Free to all members of the Garden. To others, 35 cents a copy, $2.00 a year. Now in its third volume. A continuation of the Journal of The New York Botanical Garden, fifty-one volumes.

Brittonia. A series of botanical papers. Subscription price, $7.50 per volume. Now in its eighth volume.

NEW YORK BOTANICAL GARDEN
Bronx Park, New York 58, N. Y.